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Petition for Determination of Nonregulated Status for Innate™ Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise: Events E12 and E24 (Russet Burbank); F10 and F37 (Ranger Russet); J3, J55, and J78 (Atlantic); G11 (G); H37and H50 (H)

We submit this petition under 7 CFR 340.6 to request that the Administrator make a determination that the articles should not be regulated under 7 CFR Part 340.

Submitted by Pete Clark Ph.D.
J.R. Simplot Company

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Petition for Determination of Nonregulated Status

The undersigned submit this petition under 7 CFR 340.6 to request that the Administrator make a determination that the articles should not be regulated under 7 CFR part 340.

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Release of Information

J.R. Simplot Company is submitting the information in this assessment for review by the USDA as part of the regulatory process. By submitting this information, J.R. Simplot Company does not authorize its release to any third party except to the extent it is requested and required under the Freedom of Information Act, 5 U.S.C., paragraph 552; USDA complies with the provisions of FOIA and USDA's implementation regulations (7 CFR Part 1.4). Except in accordance with the Freedom of Information act, J.R. Simplot Company does not authorize the release, publication or other distribution of this information without J.R. Simplot Company's prior notice and consent.

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Abbreviations, Acronyms, and Definitions

Abbreviation	Definition
AGP	pAgp-derived probe used in DNA gel blot hybridization
ALA	Alanine
A. tumefaciens	*Agrobacterium tumefacians*
ARG	Arginine
ASN	Asparagine
ASN1	*Asn1* gene-derived probe used in DNA gel blot hybridization
Asn1	Asparagine synthetase-1 gene
ASP	Aspartic acid
Backbone DNA	DNA associated with vector backbone
chs	Chalcone synthase gene
cwt/A	Unit of measure equal to 100lbs/ acre
CYS	Cysteine
DNA	Deoxyribonucleic acid
DNA insert	The DNA sequence from pSIM1278 located between the LB and RB intended to be integrated into the potato genome
EB	Ethidium bromide
fASN1	Fragment of the *Asn1* gene
FDA	Food & Drug Administration
G0	First generation greenhouse-grown tuber seed
G1	First generation field-grown tuber seed
G2	Second generation field-grown tuber seed
G3	Third generation field-grown tuber seed
GBS	pGbss-derived probe used in DNA gel blot hybridization
GLN	Glutamine
GLU	Glutamic acid
GLY	Glycine
GM	Genetically modified
gus	β-glucuronidase gene
Hd	Restriction enzyme *Hin*d III
HIS	Histidine
IPD	Inter-genebank Potato Database
ipt	Isopentyltransferase gene – produces cytokinin hormones associated with plant growth and development
ILE	Isoleucine
LB	Left Border (a 25-base pair sequence) similar to *A. tumefaciens* T-DNA border
Left Border side	The region of the DNA insert in the vicinity of the Left Border, which may either extend up to the cleavage site in the Left Border or be truncated at a position of between a single base pair and 100s of base pairs downstream from that cleavage site
Left Border site	The 25-base pair sequence defined as Left Border, similar to *A. tumefaciens* T-DNA

Abbreviations, Acronyms, and Definitions (Continued)

Abbreviation	Definition
LEU	Leucine
LYS	Lysine
MAFF	Japan Ministry of Agriculture, Forestry, and Fisheries
MET	Methionine
MHLW	Japan Ministry of Health, Labor and Welfare
Non-coding DNA	DNA not coding for RNA that is translated into protein
ORFs	Open reading frames
pAgp	Promoter of the ADP glucose pyrophosphorylase gene
PCR	Polymerase chain reaction
pGbss	Promoter of the granule-bound starch synthase gene
PHE	Phenylalanine
PhL	Phospholrylase-L gene
PPO	*Ppo5* gene-derived probe used in DNA gel blot hybridization
Ppo5	Polyphenol oxidase-5 gene
Pro	Functionally-active promoter
PRO	Proline
R1	Water dikinase R1 gene
RB	Right Border (a 25-base pair sequence) similar to *A. tumefaciens* T-DNA border
Right Border side	The region of the DNA insert in the vicinity of the Right Border, which may either extend up to the cleavage site in the Right Border or be truncated at a position of between a single base pair and 100s of base pairs upstream from that cleavage site
Right Border site	The 25-base pair sequence defined as Right Border, similar to *A. tumefaciens* T-DNA
RNA	Ribonucleic acid
RNAi	RNA interference
SER	Serine
Sol t 1	42 kDa allergen known as patatin
Somaclonal variation	Genetic and/ or phenotypic variation among clonally propagated plants of a single donor clone; generated by tissue culture and other forms of vegetative propagation
TPS	True potato seed
T-DNA	Transfer DNA from *A. tumefaciens* delineated by left and right border sequences
THR	Threonine
TRP	Tryptophan
TYR	Tyrosine
USDA-APHIS	United States Department of Agriculture-Animal and Plant Health Inspection Service
VAL	Valine

Summary

The J. R. Simplot Company has pioneered a new approach that marks a significant and vital advance in plant breeding. Simplot's Innate™ technologies allow us to transform plants with plant DNA . In the present petition, Innate™ technologies have been used to transform potato plants with non-coding potato DNA (DNA not coding for RNA that is translated into protein). We seek nonregulated status of potato plants transformed using our Innate™ technologies and submit the evidence that these plants should not be classified as "Regulated articles" as defined under 7 CFR 340.

To explain, Simplot's Innate™ technologies allow researchers to isolate genetic elements from any plant genome, rearrange them, or link them together in desired permutations, and introduce them back into the genome. More specifically, in these events, the genomic DNA comes from potato or wild potato (a group of related plant species that are sexually-compatible with potato). We incorporate no viral markers, and no vector backbone sequences, into the plant genome.

As demonstrated in the data presented in this petition, the transformed cultivated potato plants are not parasitic, and our data show no increase in pest potential compared to the controls. Furthermore, the expression of the integrated genetic elements results in traits highly sought-after in the potato industry, namely: reduced black spot bruise, low asparagine, and lower levels of reducing sugars. One key consequence of these beneficial traits is that the harvested potatoes contain less acrylamide when cooked than untransformed, commercially available potatoes.

Among other things, we have used Innate™ technologies to address two critical potato quality issues: (1) large amounts of asparagine, a non-essential free amino acid that is rapidly oxidized to form acrylamide upon frying or baking; and (2) susceptibility to enzymatic browning and discoloration, which happens when polyphenol oxidase leaks out from the damaged plastids of bruised potatoes. In the cytoplasm, the enzyme oxidizes phenols, which then rapidly polymerize to produce dark pigments. Browning is also triggered non-enzymatically as a consequence of the partial degradation of starch into glucose and fructose. When heated, these reducing sugars react with amino acids, such as asparagine, through the Maillard reaction to produce a variety of desired compounds contributing to flavor, aroma, and browning, but also acrylamide.

Because our Innate™ technologies effectively accelerate the process of conventional crossing, it allows desired changes in traditional varieties to occur much faster than is currently possible, while maintaining the desired characteristics of the original parent plant. For potatoes, Innate™ technologies are particularly attractive because potatoes are notorious for having a high degree of heterozygosity, suffering from inbreeding depression, and limited predominantly to clonal propagation. These inherent factors significantly hinder and prevent us from commercially introducing quality, sought-after traits into this valuable crop. Today's traditional breeding techniques simply result in random genomic rearrangements and trait segregation, and do not allow for the simultaneous addition of multiple desired traits.

To illustrate this point, there currently are no varieties available that produce tubers with low acrylamide potential, reduced black spot bruise and reduced sugars, while displaying all other traits important to the food industry. Therefore, instead of attempting to develop new varieties, the J. R. Simplot Company improved the quality of five existing potato varieties by transforming them with a non-coding transfer DNA that silences the genes related to expression of black spot bruise, asparagine,

and reducing sugars in tubers. Our proprietary plant expression cassettes, included in the DNA insert, are derived from the potato genome.

This DNA insert comprises two expression cassettes and was inserted into our pSIM1278 transformation vector. The first cassette comprises fragments of both the asparagine synthetase-1 gene (*Asn*1) and the polyphenol oxidase-5 gene (*Ppo5*), arranged as inverted repeats between the Agp promoter of the ADP glucose pyrophosphorylase gene (*Agp*) and the Gbss promoter of the granule-bound starch synthase gene (*Gbss*) and results in silencing of both the *Ppo5* and *Asn1* genes. The second cassette is comprised of fragments of the promoters of the starch associated gene (*R1*) and the phosphorylase-L gene (*PhL*), operably linked to the same Agp and Gbss promoters as the first cassette. The function of the second cassette is to silence the promoters of the starch associated gene (*R1*) and the phosphorylase-L gene (*PhL*). Importantly, in keeping with our Innate™ technologies, these expression cassettes contain potato genomic DNA, *e.g.* DNA from either the selected plant species or from a plant that is sexually compatible with the selected plant species.

We transformed five different varieties including Ranger Russet, Russet Burbank, Atlantic, variety G, and variety H with pSIM1278, and identified transformed shoots using polymerase chain reaction (PCR) rather than markers. We also performed numerous tests and confirmed that none of the plants contained vector backbone DNA. Transformed plants were propagated to produce the Innate™ events. Ten of these events were selected for detailed analyses: F10 and F37 for Ranger Russet, E12, and E24 for Russet Burbank, J3, J55, and J78 for Atlantic, G11 for variety G, and H37 and H50 for variety H. See **Table 1** for their OECD unique identifiers.

Table 1. Innate™ events and their OECD unique identifiers

Event number	OECD Unique Identifier
F10	SPS-∅∅F10-7
F37	SPS-∅∅F37-7
E12	SPS-∅∅E12-8
E24	SPS-∅∅E24-2
J3	SPS-∅∅∅J3-4
J55	SPS-∅∅J55-2
J78	SPS-∅∅J78-7
G11	SPS-∅∅G11-9
H37	SPS-∅∅H37-9
H50	SPS-∅∅H50-4

Extensive analyses of the various events demonstrated that the achievement of the desired modified traits was enabled by targeted gene silencing. Simplot found there to be reduced RNA (transcript) levels of the four targeted genes in tubers, which was found to be associated with:

(1) 69 – 78 percent reductions in free-asparagine;
(2) Black spot bruise tolerance; and
(3) Lower levels of reducing sugars.

Eight of the selected events contained both expression cassettes. Two events, J78 and G11, had only the silencing cassette for the *Asn1* and *Ppo5* genes integrated into their genomes and produced tubers with successfully down-regulated expression of the *Asn1* and *Ppo5* genes. The effective reduction of

asparagine found in events J78 and G11, resulting from silencing of the *Asn1* and *Ppo5* genes results in significantly reduced acrylamide (66 to 70%), making these commercially valuable potato varieties even without silencing the starch associated genes (*R1*) and phosphorylase-L (*PhL*).

Ultimately, we demonstrate that the Innate™ tubers exhibited the desired traits which can lead to lower acrylamide levels in cooked potatoes and, importantly, we showed that the transformed tubers were otherwise substantially equivalent to untransformed control tubers.

The propagation of commercial potato varieties through cloning of seed potatoes mitigates concerns about increased weediness or pest potential such as seed dispersal, survival outside of cultivation, or outcrossing. One of the most important potato varieties, Russet Burbank, produces few flowers and is male sterile. Other factors limiting outcrossing include the tendency for most fertile varieties to be self-pollinated, an inability to attract honey bees because they lack nectar, the pollen transfer range is limited to about 20 meters, and true seeds would be unlikely to grow into mature potatoes since potato seeds are not saved and propagated in the typical farming operation. If potatoes were grown from true potato seed, the offspring would be so diverse that they would not be useful as commercial potatoes. In addition, potatoes are not known to escape from commercial fields or show weediness potential. Wild potato varieties are rare in the United States and for the most part geographically isolated from commercial production areas, further reducing concerns about cross-pollination with wild species.

The modifications described in this petition were intended to enhance the quality, not agronomic characteristics, of potato by inactivating four native genes. Planting, cultivation, management and harvesting techniques were not affected by the incorporated traits. The Innate™ varieties are likely to be planted in areas that are already growing potatoes, and would not result in a significant expansion of acres. The modifications described in this petition are highly unlikely to increase the weediness or invasiveness of potato because the incorporated traits (reduced free-asparagine, black spot bruise tolerance, and reduced sugars) do not play a role in survival, dispersal, or ecological competitiveness. Field trials over multiple years with the 10 events did not provide any evidence for altered growth characteristics such as accelerated tuber sprouting, increased plant vigor, increased tuber set, or delayed senescence. No new pesticides are expressed, and no natural defense mechanisms activated, and thus modified potatoes are unlikely to display enhanced weediness or pesticidal properties. Therefore, the potential to impact insects and other non-target organisms, weed or disease susceptibility, endangered species or biodiversity is negligible for Innate™ potatoes.

We therefore seek nonregulated status for these events based on the weight of evidence demonstrating their safety when compared to untransformed potatoes. The technologies presented in this petition and the resultant transformed plants and their tuber products satisfy the requirements for nonregulated status. The transformed plants and subsequent traits described in this petition should not be considered plant pests. In conclusion, plants displaying the incorporated traits which have resulted from the addition of well characterized, non-coding regions from potato or wild potato are as safe as untransformed potatoes based on all of the data contained in this submission. The J.R. Simplot Company requests a determination from APHIS that the 10 potato events described in this petition, and any progeny derived from these events, are granted nonregulated status and would no longer be considered regulated articles under 7 CFR Part 340.

The genetic services and research and development methods and associated genetic tools, disclosed in this petition, such as expression vectors, and the transformed plants and plant cells, and methods of transformation, have been trademark-branded by Simplot as Innate™.

1. Rationale for Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise

The potato varieties were transformed using Innate™ technologies in order to address the need of the potato industry to improve quality by reducing expression of the enzyme responsible for black spot bruise and to reduce acrylamide through lowering the concentration of the reactants, namely asparagine and reducing sugars. Use of Innate™ technologies allows for transformation of potatoes using genetic material that contains only non-coding regulatory regions. The desirable traits were simultaneously incorporated into five of the most popular potato processing varieties. Such trait addition into existing varieties would not be possible to achieve through traditional breeding because potato is tetraploid, highly heterozygous and sensitive to inbreeding depression. The resulting Innate™ potatoes are not plant pests as verified by phenotype, Southern blot hybridization, and PCR.

1.1. Basis for Determination of Nonregulated Status

Part 340 Section 7 of the United States Federal Register regulates, among other things, the introduction of organisms and products altered or produced through genetic engineering that are plant pests or are believed to be plant pests. The 10 events described in this petition were transformed with DNA that does not alter the pest characteristics of the potatoes. All events were confirmed to be free of *Agrobacterium* and free of *Agrobacterium*-derived backbone DNA. In addition, the scientific evidence presented here shows that the inserted genetic material contains only non-coding regulatory regions and are as safe as untransformed potatoes.

Field evaluations demonstrated that the events displayed similar agronomic and phenotypic characteristics compared to their untransformed controls. This submission reviews the biosafety implications of all minor differences observed. The most important intentionally-incorporated trait is a reduced level in the free amino acid, asparagine in tubers. As predicted, we confirmed that this change was associated with a reduced potential to form acrylamide upon frying.

Most of the events also displayed lowered levels of the reducing sugars glucose and fructose when fresh or when analyzed at one month after harvest (**Appendix 9. Compositional Analyses**). Although reduced asparagine alone significantly decreases acrylamide formation, as shown in events J78 and G11, the lowered levels of reducing sugars further reduces acrylamide formation and limits heat-induced browning. The plant-derived transfer DNA used for transformation consisted of two inverted repeats. Each of these repeats was inserted between promoters that are predominantly active in tubers. Expression of the first inverted repeat triggered the degradation of transcripts for the asparagine synthetase-1 (*Asn*1) and the polyphenol oxidase-5 (*Ppo5*) genes. Expression of the second inverted repeat resulted in reduction in the starch associated gene (*R1*) and the phosphorylase-L gene (*PhL*) transcripts in tubers. The events presented in this submission all contained at least the fragment of the DNA insert carrying the *Asn1*/*Ppo5* gene silencing cassette.

The 10 events produced by transformation of 5 potato varieties with the specified non-coding transfer DNA are well characterized and safe as determined by agronomic and compositional evaluations, when compared to the untransformed controls.

1.2. Rationale for Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise

The Swedish National Food Administration and Stockholm University announced in April 2002, a relationship between the formation of certain blood adducts and the consumption of foods later found to contain acrylamide (NTP 2012). Other researchers also studied the mechanism for acrylamide formation in food, and the J.R. Simplot Company had also earlier predicted that acrylamide would form as a result of the well-known but complex Maillard browning reaction. Cooking or heat treating of foods that are rich in reducing sugars and amino acids may result in browning, commonly referred to as the Maillard reaction. This reaction occurs between sugars and amino acids, affecting changes in the color, flavor, functional properties and nutritional value of food (Obrien and Morrissey 1989). More specifically, we found acrylamide formed primarily when the amino acid asparagine along with reducing sugars were heated at temperatures above 120°C, as would occur during frying and baking. The biochemical basis of acrylamide formation was later published by Stadler *et al.* (2002).

Various governments responded to the news about finding acrylamide in food by providing guidance and surveys. Through one such survey, the FDA determined that potato products contribute 35% of the acrylamide exposure through diet in the US. The scientific community has since gathered additional information, including epidemiology and toxicology studies. Along with studies to mitigate the levels of acrylamide in food, the scientific community has also been working to understand the health implications of acrylamide consumption (NTP 2012).

Based on a recent notice (Federal Register 2009), FDA is considering issuing guidance for industry on the reduction of acrylamide levels in food products. The introduction of Innate™ potatoes with low acrylamide potential would provide potatoes that are largely indistinguishable from existing varieties. The reduction in asparagine and sugars using Innate™ technologies and the resulting reduction in acrylamide upon heating will address food industry needs with respect to the proposed FDA guidance. As such, this approach to lowering acrylamide could be adopted readily by the food industry if such guidance is issued by the FDA. In addition, litigation in the state of California as a result of Proposition 65 (OEHHA 2011a; OEHHA 2011b) resulted in legal settlements with restaurant chains and the retail french fry and potato chip manufacturers regarding perceived risks from acrylamide. Some settlements required signs warning consumers, but in addition, some manufacturers of retail french fries and potato chips would be required to reduce acrylamide in their products or add warning labels. As of December 1, 2011, some potato chip manufacturers began including a statement on packaging warning consumers of the presence of acrylamide and its relationship to browning in potatoes. Potato processors affected by these rulings in California could be motivated to adopt the low acrylamide, Innate™ potato products.

Following the discovery of acrylamide in foods and in response to governmental concerns, many additives were studied and some have shown effectiveness at reducing acrylamide. Some of the more promising options include citric acid, enzymes such as asparaginase, and amino acids that substitute for asparagine in the Maillard reaction and therefore form products other than acrylamide when heated.

Another approach to reducing acrylamide could be based on either traditional breeding or biotechnology to reduce the precursors for acrylamide. The use of biotechnology allows for specific deactivation of genes involved in the formation of asparagine and reducing sugars. In a crop like potatoes, which is highly heterozygous and suffers from inbreeding depression, the speed to develop such changes could take decades through traditional breeding. In contrast, biotechnology allows for specific changes in traditional varieties much faster, while maintaining the desired characteristics of the original parent.

Potato transformation with the pSIM1278 construct results in the incorporation of two silencing cassettes into the potato. Expression of the first cassette lowers transcript levels for the *Asn1* (asparagine synthetase-1) and *Ppo5* (polyphenol oxidase-5) genes and, consequently, limits (1) formation of the acrylamide precursor asparagine, and (2) formation of impact-induced black spot bruise that occurs when the enzyme polyphenol oxidase oxidizes phenols to produce dark pigments. The presence of black spot bruise results in lower quality and subsequent production losses during processing into fries or chips. A reduction in the formation of reducing sugars is accomplished by the down-regulated transcript levels for the *PhL* (phosphorylase-L) and *R1* (starch associated) genes resulting from expression of the second cassette. These traits function by slowing the conversion of starch to reducing sugars (glucose and fructose). Benefits include improved quality, especially relating to color control, and thus contributing to the desired golden brown colors required by most french fry or chip customers. Also, the reducing sugars react with amino acids, such as asparagine, to produce Maillard products including acrylamide.

1.3. Benefits of Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise

The events described here produce tubers with a greatly reduced potential to form acrylamide, thus addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

Many methods have been tested and research is ongoing to reduce acrylamide through process changes, reduction in dextrose, and additives such as asparaginase, citrate, and competing amino acids. The required capital expense to implement process changes throughout the potato industry would cost millions of dollars. In addition to the expense, these process changes have significant drawbacks including potentially negative flavors associated with additives such as asparaginase or citrate. Typically, fry manufacturers add dextrose during processing of french fries to develop the desired golden brown color, but dextrose also increases the formation of acrylamide through the Maillard reaction. Significant reductions in acrylamide occur by merely omitting dextrose from the process; however, the signature golden brown colors must then be developed some other way (such as though the addition of colors like annatto). The use of alternate colors, results in an absence of the typical flavors that develop through those browning reactions. Another challenge with the use of additives to reduce reactants like asparagine will be the principle of moisture migration that occurs during frozen storage resulting in a return of asparagine to the surface and increased acrylamide compared with the initial testing at the time of processing. In contrast, the Innate™ potatoes will significantly reduce acrylamide without the use of new additives or process changes. The resulting foods will have the same appealing color, flavors, and aromas as they currently have today.

The blackening that occurs after potatoes are bruised affects quality and recovery in processing french fries and chips. Potatoes that have been damaged and show black spot bruise must be trimmed or could be rejected before processing, resulting in quality challenges or economic loss. Management of reducing sugars presents significant challenges and potential financial loss because processors reject loads with reducing sugar content above 2%, which is approximately 20% of potatoes produced. However, potatoes developed with Innate^{TM} technologies exhibit significantly less black spot bruising and lower reducing sugar content, which will likely have economic benefits for growers and processors.

1.4. Submissions to Other Regulatory Agencies

Simplot has initiated and will complete a consultation process with the Food and Drug Administration (FDA) prior to commercial distribution of tubers or products from the 10 events.

An assessment of the safety of these events will also be submitted to Health Canada and the Canadian Food Inspection Agency (CFIA) in 2013. Furthermore, submissions in support of approval to import products from the events will be made to the Japan Ministry of Health, Labor and Welfare (MHLW) and the Ministry of Agriculture, Forestry, and Fisheries (MAFF). Submissions are anticipated for other key international markets including Mexico.

1.5. Conclusions: Rationale for Development of Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise

In summary, there is an important need to improve the quality and reduce levels of acrylamide in fried and baked potatoes. Because it was not feasible to reduce acrylamide formation and black spot bruise in existing varieties through traditional breeding, we accomplished this goal by applying Innate™ technologies. The varieties represented in this petition represent almost 30% of the US Potato market. However, about 70% of the remaining acreage and over 70 major varieties could benefit from the reduction in acrylamide through transformation with pSIM1278. We now seek nonregulated status for 10 events based on the weight of evidence demonstrating their safety. We have transformed plants with the addition of genomic DNA from potato and wild potato where the integrated genetic material contains only well characterized non-coding regulatory regions that are as safe as untransformed potatoes.

2. The Biology of Potato

This section is based, in part, on the Organization for Economic Co-Operation and Development's (OECD) series on harmonization of regulatory oversight in biotechnology no. 8, Consensus document on the biology of Solanum tuberosum, 1997.

This section describes potato biology and how it contributes to the effective management of the Innate™ potatoes. All commercial potato varieties must be propagated through cloning, effectively mitigating many concerns about increased pest potential such as seed dispersal, survival outside of cultivation, or outcrossing.

2.1. History of Potato

The cultivated potato traces its origin to Andean and Chilean landraces developed by pre-Colombian cultivators. These landraces exhibit morphological and genetic diversity, and are distributed from western Venezuela to northern Argentina, and in southern Chile (Spooner *et al.* 2005). Introduced to Europe by Spain in 1536, the potato was subsequently conveyed by European mariners to territories and ports throughout the world. Once established in Europe, the potato soon became an important food staple and field crop. But lack of genetic diversity, due to the fact that very few varieties were initially introduced, left the crop vulnerable to disease. In 1845, a plant disease known as late blight, caused by the fungus-like oomycete pathogen *Phytophthora infestans*, spread rapidly through the poorer communities of western Ireland, resulting in the crop failures that led to the Great Irish Famine. Potato remains an essential crop in Europe, where per capita production is still the highest in the world, but the

crop also made its way back over the Atlantic Ocean to North America. The industry gained greater importance particularly in western states after the horticulturist Luther Burbank developed the Russet Burbank potato in 1872. In 2011, the United States harvested 21.4 million tons of potatoes, enough to make it the world's fourth biggest producer (NPC 2012). Potatoes in the United States are grown in many states, although about half of the crop comes from Idaho and Washington. The top ten potato producing states of Idaho, Washington, Wisconsin, North Dakota, Colorado, Oregon, Maine, Minnesota, California and Michigan account for almost 88% of the United States potato crop (USDA-NASS 2010b). Most potatoes are harvested in July through October. Only about one third of US potatoes are consumed fresh. Around 60% of annual output is processed into frozen products (such as frozen fries and wedges), chips, dehydrated potato and starch, while 6% is replanted as seed potato. Americans eat, on average, approximately 54 kg of potatoes per person per year. The most rapid expansion in production and consumption of potatoes has occurred in southern and eastern Asia. China is now the world's largest potato-producing country, and nearly a third of the world's potatoes are harvested in China and India.

2.2. Use of the Potato as Food and Feed in the USA

Harvested potatoes are either used for food (~93%), feed (<1%), industrial purposes (<1%) or as “seed” for planting (5%). The type of use is partially dependent on a variety of characteristics related to tuber quality. According to the National Potato Council, approximately 50% of all tubers are used for the combination of frozen fries, potato chips, and shoestrings (julienne cut crispy snacks), whereas 11% are dehydrated and <2% are canned. An additional 28% of tubers are not processed but sold as fresh potatoes (NPC 2012).

Raw potato waste products (peels, out of specification raw potatoes, or other non-processed raw potato products) and processed discards (french fry, hash brown, etc.) are routinely incorporated into feed rations at livestock feedlot operations including those owned by the J.R. Simplot Company. Approximately 15,000 tons of processed potato waste and 60,000 tons of raw potato waste are integrated into livestock feed at the J.R. Simplot Company’s Grandview, ID feedlot on an annual basis. The typical feed ration used for cattle finishing at J.R. Simplot Company feedlots includes 8-14% potato waste. One of the major benefits of using the potato waste is that it is not discarded into landfills, but used in a sustainable manner as part of a long-term approach to reducing waste and integrating sustainability with respect to the Earth’s valuable natural resources.

2.3. Taxonomy of the Genus *Solanum*

The *Solanaceae* family contains several well-known cultivated crops such as tomato (*Solanum lycopersicum* also referred to as *Lycopersicon esculentum*), eggplant (*Solanum melogena*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*) and potato (*Solanum tuberosum*). Within the genus *Solanum*, over a thousand species have been recognized. Potatoes will not hybridize with non-tuber bearing *Solanum* (tomato, eggplant, etc.) species including weeds commonly found in and around commercial potato fields (Love 1994).

The genus *Solanum* is divided into several subsections, of which the subsection *potatoe* contains all tuber-bearing potatoes. The subsection *potatoe* is divided into series, of which *tuberosa* is relevant to this document. Within the series *tuberosa* approximately 54 species of wild and cultivated potatoes are found. One of these is *S. tuberosum*.

S. tuberosum is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum* is the cultivated potato widely in use as a crop plant in, for example, North America and Europe. The subspecies *andigena* is also a cultivated species, but cultivation is restricted to Central and South America (Hanneman 1994).

2.4. Genetics of Potato

The basic chromosome number in the genus Solanum is twelve. *S. tuberosum* subsp. *tuberosum* can be diploids (2n=2x=24) or tetraploids (2n=4x=48). The diploids have a limited range in parts of South America, while the tetraploids are the most commonly cultivated all over the world. How tetraploidy originated in potato is unclear. The cultivated *S. tuberosum* subsp. *tuberosum* can be either an autotetraploid (doubling of the chromosomes of a diploid species) or an allotetraploid (doubling of the chromosomes of a diploid hybrid between two related species).

While nearly all diploid species are self-incompatible, the cultivated tetraploid *S. tuberosum* subsp. *tuberosum* is capable of self-pollination (selfing). Plaisted (1980) has shown that under field conditions selfing is most likely for tetraploid *S. tuberosum,* with 80-100 percent of the seeds formed due to selfing. Conner and Dale (1996) collected outcrossing data from several field experiments with genetically modified potatoes, performed in New Zealand, the United Kingdom and Sweden. In each study, the outcrossing rate was zero when receiving plants were separated by more than 20 meters from the genetically modified ones. Although many Solanum species are fertile, it appears that a large number of the tetraploid cultivated *S. tuberosum* subsp. *tuberosum* cultivars have reduced fertility. Events of three of five varieties in this petition produce abundant flowers and pollen. However, Russet Burbank and the H varieties and events are sterile and have no outcrossing potential.

2.5. Potato Growth and Life Cycle

Potatoes grown in both commercial and organic farming operations go through the following five general growth stages of development (Dwelle 2003): 1) Sprouts develop from eyes on seed tubers, grow upwards, and emerge from the soil. Roots initiate development at the base of the emerging sprouts, 2) Vegetative growth begins. Leaves and branch stems develop from aboveground nodes. Roots and stolons develop at belowground nodes, 3) Tubers begin to form at stolon tips, but do not enlarge. Flowering is initiated at the end of this stage, 4) Tuber cells expand with the accumulation of water, nutrients, and carbohydrates, and 5) Vines turn yellow and lose leaves, tuber growth slows and vines die. Tuber maturation is completed as tuber skins set.

In greater detail, the potato life cycle begins with certified seed potatoes, which are cut into pieces containing at least one eye and are typically planted in the field from February through May. Plants will establish by forming vegetative tissues, including roots, leaves, stems, and stolons. Tuber formation begins approximately 30-60 days after planting. Tubers are derived from lateral underground buds that develop at the base of the main stem, when kept underground. These develop into stolons due to diagravitropical growth. When conditions are favorable for tuber initiation, the elongation of the stolon stops, and cells located in the pith and the cortex of the apical region of the stolon first enlarge and then divide longitudinally. The combination of these processes results in the swelling of the subapical part of the stolon. During enlargement, tubers become the largest nutrient sink of the potato plant storing large amounts of carbohydrates (mainly starch) and also significant amounts of protein. Furthermore, potato tubers decrease their general metabolic activity and as such behave as typical storage sinks. Potato tubers of varieties such as Ranger Russet, Russet Burbank, Atlantic, G, and H are harvested from 90 to

160 days after planting, which may vary with production area and marketing conditions. Typical potatoes are about 20-24% dry matter, of which approximately 60-80% consists of starch. After potato vines die back, the tuber skins thicken and harden which provides greater protection to tubers during harvest and blocks entry of pathogens. Although dormancy is defined as the absence of visible growth, dormant tuber meristems are metabolically active. In general, rates of many cellular processes such as respiration, transcription, and translation are suppressed during dormancy and non-dividing, dormant tuber meristems are arrested in the G-1 phase (a period prior to the synthesis of DNA in mitosis). Activation of cell metabolism during early spring triggers the development of sprouts from the eyes of the primary tuber.

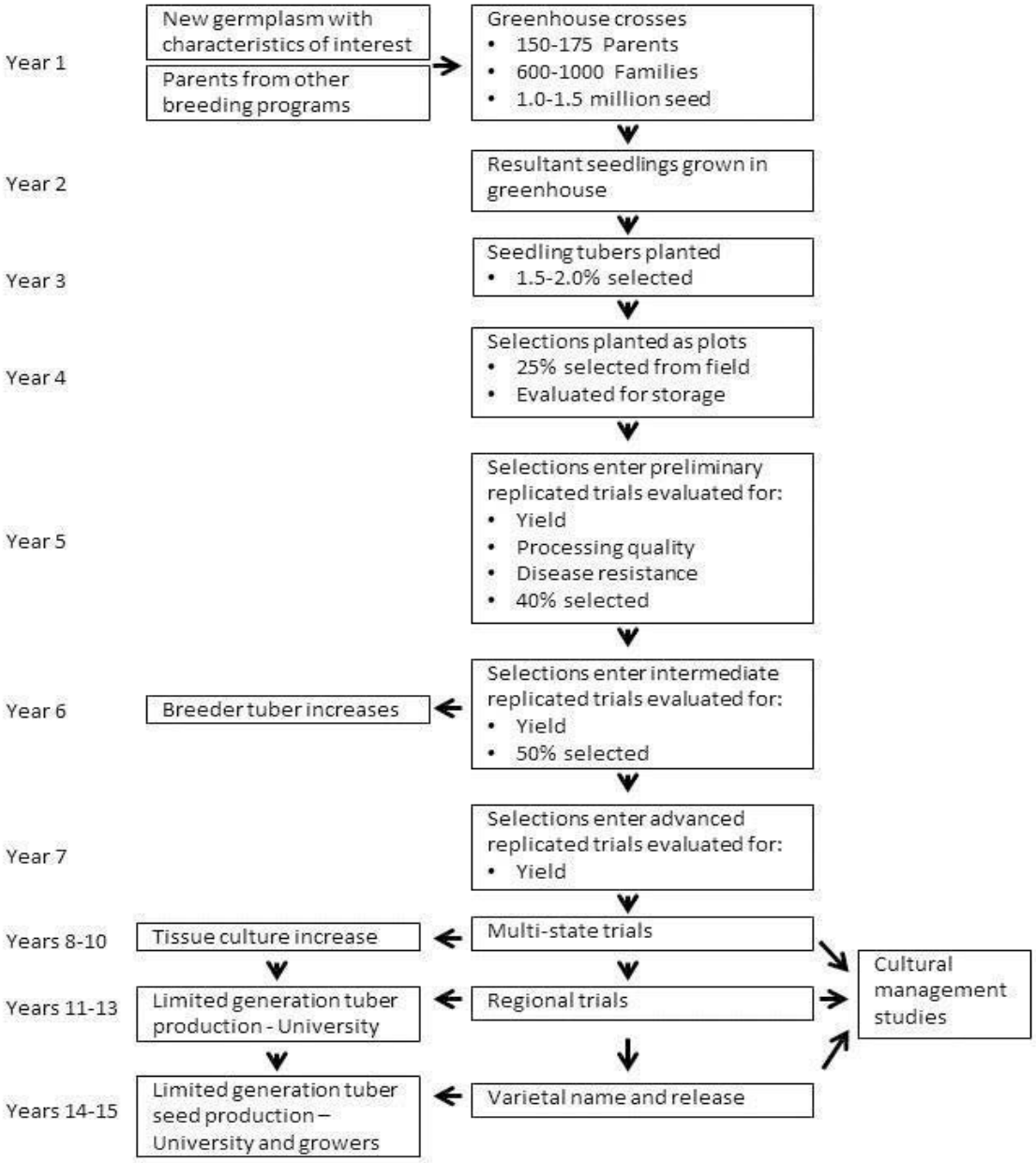
2.6. Seed Propagation

Potato is clonally propagated, which means that tubers rather than seeds are used for planting. A single potato plant produces approximately 12 tubers, therefore it takes multiple years to propagate a new potato variety and generate sufficient amounts of tuber seed needed for commercialization. A typical seed bulk-up program would progress as follows. In the initial year, propagation in tissue culture would produce about 100 plants, and then these plants are grown in a greenhouse to produce thousands of mini-tubers, also called “nuclear seed”. In the second year, mini-tubers are planted at field sites with the lowest possible incidence of diseases and pests, producing generation-1 (G1) seed. Those G1 tubers are grown in the third year to increase the amount of tubers, producing G2 seed. In the fourth, fifth, and sixth years, bulk-up continues with commercial seed often representing G3, G4, and G5 tubers respectively.

2.7 Variety Development

Potato varieties take many years to develop (see **Figure 1**). The decision to establish a new variety is based on many factors such as need in the market place, potential consumer acceptance, and pest tolerance or resistance. Potato varieties do not have a high frequency of introduction and discontinuation compared to some other crops such as field corn or soybeans. Since potatoes are clonally propagated, there is a reduced risk of varietal dilution due to cross pollination.

Figure 1. Potato Variety Development Schematic



2.8. Recipient Potato Varieties

The potato varieties chosen for modification all represent significant value to the potato industry and relatively large percentage of the overall acreage (**Table 2**).

Table 2. Recipient Potato Material

Variety	Usage	Percent of Seed Acreage in US[1]
Russet Burbank	Fries	22.0
Ranger Russet	Fries	6.3
Atlantic	Chips	2.6
G	Chips	2.0
H	Chips	0.7

[1]2009 data for seed potatoes: http://www.nationalpotatocouncil.org/NPC/resources_statistics.cfm

2.9. Typical Agronomic Practices

Examples of typical agronomic practices for a successful potato crop are described in **Table 3**.

Table 3. Example of Agronomic Inputs for Two Potato Variety Classes

	Russet Varieties	Round White Varieties
Planting Date	April 1 to May 10	April 1 to May 10
Planting Rate	15,000 - 18,000 seed pc or 17 – 23 cwt/A	17,000 – 19,000 seed pc or 19 – 24 cwt/A
Row Spacing	34-36” between rows	34-36” between rows
Seed Spacing	10-12” within row	8-10” within row
Fertilizer	For 600 cwt/A yields and optimum soil test levels: 250 lb N – 100 lb P_2O_5 – 330 lb K_2O per acre	For 600 cwt/A yields and optimum soil test levels: 250 lb N – 100 lb P_2O_5 – 330 lb K_2O per acre
Yield/Plant	2-4 lb	2-4 lb
Yield/Acre	400-700 cwt/A	400-700 cwt/A
Harvest Date	September 1 to October 15	August 1 (early season varieties only) to October 15

2.10. Pollination and Outcrossing

Potato is clonally propagated, which means that tubers rather than seeds are used for planting. Harvested tubers are either used for food (~90%), feed (<5%), industrial purposes (<2%) or as “seed” for planting (5%). Therefore pollination to produce seed is not a factor in major commercial potato production, *e.g.*, if Innate™ potatoes were planted in close proximity to organic potatoes, any cross pollination would not impact the organic tubers.

Some of the most important potato varieties including Russet Burbank, produce few flowers and are male sterile. The variety H produces few if any flowers and exhibits male infertility. Many cultivars flower less than wild material, and flowers that do form often drop after pollination resulting in no berry formation. Flowering has no impact on tuber development.

Approximately 80% of seed produced by fertile varieties is derived from self-pollination (Plaisted 1980). Cross-pollination is typically mediated by certain insects including bumblebees (*Bombus* spp.) that

typically don't travel much more than 3 kilometers, rather than wind pollination (OECD 1997). Additionally, other bee species such as honey bees (*Apis mellifera*) are not pollinators of potatoes since potato flowers do not contain ample nectar (OECD 1997). Field evaluations in New Zealand, the United Kingdom and Sweden demonstrated through genetic testing that outcrossing rates were zero when receiving plants were separated by more than 20 meters from genetically modified plants (Conner and Dale 1996). Additionally, potato pollen grains are extremely temperature sensitive. Pallais *et al.* (1988) showed that pollen viability decreased by 30 to 70% when exposed to temperatures of 30°C for up to 30 minutes.

2.11. Wild potatoes in the U.S.

This discussion is based on data in the U.S. Potato Genebank and on a monogram published by the American Society of Plant Taxonomists entitled Wild Potatoes (*Solanum* section *Petota; Solanaceae*) of North and Central America (Spooner *et al.* 2004). The USDA maintains the US Potato Genebank and participates in the Association of Potato Inter-genebank Collaborators (APIC), which has produced a global inventory of wild potato genetic resources available on the internet, the Inter-genebank Potato Database (IPD 2011), which includes the U.S. Potato Genebank records. Many of the IPD records from the U.S. were obtained by John Bamberg and associates (including Spooner) during a decade of collecting wild potatoes in the Southwest U.S. (Bamberg *et al.* 2003).

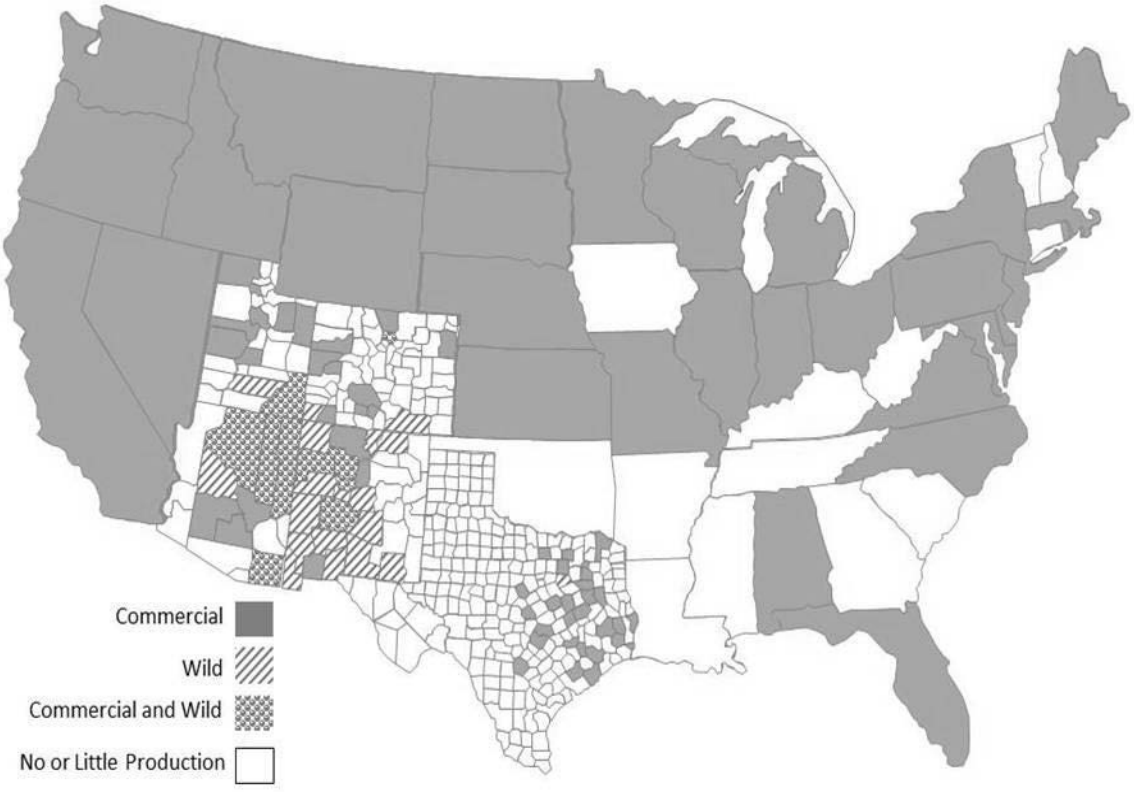
The only two wild potato species that grow within the borders of the USA, and for which specimens exist in gene banks, include the tetraploid species *S. fendleri* (recently reclassified as *S. stoloniferum*; however, some sources, including the IPD, still use the *S. fendleri* designation) and the diploid species *S. jamesii* (Bamberg *et al.* 2003; IPD 2011; Bamberg and del Rio 2011a; Bamberg and del Rio 2011b; Spooner *et al.* 2004). Love (1994) reported that a third species, *S. pinnatisectum*, is also a native species in the USA. However, Spooner *et al.* (2004) determined that what was previously thought to be *S. pinnatisectum* was in fact *S. jamesii.* Through more than 10 years of field work and assessments of existing records, Bamberg *et al.* (2003) and Spooner *et al.* (2004) established the presence of only these two species, *S. fendleri* and *S. jamesii,* in the U.S. These researchers also attempted to verify previously recorded locations, and through this process, updated the maps of current known locations of these species, providing latitude and longitude locations for each documented population (Bamberg *et al.* 2003) and distribution maps (Spooner *et al.* 2004). These species mostly reside in dry forests, scrub desert, and sandy areas at altitudes of 5,000 to 10,000 feet, well isolated from most commercial production areas (Bamberg and del Rio 2011a).

While there is some overlap between the acreage used for commercial production and occurrence of wild species on a county level, the majority of the potato production in the United States is not in wild potato zones (**Figure 2**). However, there is a possibility that a few wild potato plants may be growing near potato fields (Love 1994). Spooner *et al.* (2004) describe *S. jamesii* habitat in the U.S. as among boulders on hillsides, sandy alluvial stream bottoms, in gravel along trails or roadways, rich organic soil of alluvial valleys, sandy fallow fields, grasslands, juniper-pinyon scrub deserts, oak thicket, coniferous and deciduous forests at elevations between 4,500 to 9,400 feet. They describe *S. fendleri* habitat similarly, and at elevations between 4700 to 11,200 feet. The risk of hybridization between commercial varieties and wild species is low because of the lack of geographical overlap and the fact that potatoes are grown from tuber seed. Furthermore, studies discussed in Conner and Dale (1996) indicate that a separation of 20 meters will be sufficient to prevent outcrossing between wild and commercial potatoes.

Love (1994) evaluated the risk associated with growing transgenic potatoes in Canada and the USA and concluded that, given the number and potency of barriers to hybridization and more specifically to introgression and stabilization, the only sound conclusion is that gene introgression into wild *Solanum* species will not occur under natural conditions in these geographies. Therefore, according to Love (1994), potato gene movement from commercial fields to wild potato species would not occur.

Figure 2. Potato Production in the United States

(County Information is for states with wild potato populations (Hijmans and Spooner 2001)



Based on conversations with Bamberg, Love (1994) reported that no one has ever reported finding hybrids between native and cultivated potatoes in the U.S., although gene transfer has been accomplished using special laboratory techniques (Love 1994). Love concluded that, based on the barriers that exist (including geographic isolation as described previously), endosperm imbalances, and multiple ploidy levels, natural hybridization is highly unlikely, and gene introgression (cross hybridization over multiple generations) is impossible or at least highly improbable (Love 1994). More recently, the US EPA has concluded that, based on its review of the scientific literature, successful gene introgression between native and cultivated potatoes in the U.S. is virtually excluded due to constraints of geographical isolation and other barriers to natural hybridization. These barriers include incompatible (unequal) endosperm balance numbers that lead to endosperm failure and embryo abortion, multiple ploidy levels and incompatible mechanisms that do not express reciprocal genes to allow fertilization to proceed. No natural hybrids have been observed between these species and cultivated potatoes in the U.S. (US EPA 2011). Based upon these biological barriers to hybridization, it is unlikely that inter-species pollination would occur with the Innate™ potatoes that are the subject of this petition.

2.12. Weediness

Standard growing practices for potatoes make it highly unlikely that potatoes would persist in a field from one crop cycle to the next. Where potatoes are a rotation crop, as is often the case, other crops such as alfalfa, corn or wheat would be grown following potatoes. In that situation, any potatoes left in the field would be eliminated by tilling, field preparations with herbicides, and harsh winters. In the rare event that potatoes survived mixed in with another crop, they have to face the same challenges during the next year.

Potatoes are typically grown on a 3 year minimum rotation to minimize soil-borne disease buildup such as white mold, pink rot, *Pythium* leak, *Verticillium* wilt, and powdery scab (Hopkins *et al.* 2003). Potatoes left to grow in the field are not desired as they can harbor disease, have no protection with fungicide, and could compete with the desired rotation crop the following year. For these reasons, it is standard practice for potato growers to monitor and control such growth, either with herbicide or tillage. Soil is disturbed greatly during potato growth and harvest operations, therefore requiring tillage prior to planting the rotation crop. Typically a broad spectrum pre-emerge herbicide is applied in the rotation crop to control a wide variety of weeds. Potato growers rarely leave the ground fallow following potato to maximize economic return. If potatoes grew inadvertently in the field left fallow, they would not be protected with insecticide or fungicide and would be susceptible to insects such as Colorado potato beetle and diseases such as early blight, further decreasing the chance of survival and reproduction.

Outside of cultivation, potato seedlings from tubers have difficulty establishing themselves as they cannot compete effectively with other plants (Love 1994). Potato is not known as a colonizer of unmanaged ecosystems. In climax vegetation, potatoes are not able to compete with other species such as grasses, trees and shrubs (CFIA 1996), virtually eliminating any chance for them to become feral. Furthermore, potatoes generally do not cause toxic effects to other plants or animals (including non-target organisms) making pest potential negligible.

Although almost all growers use tubers for planting, there is a small market for “true potato seed” (TPS). TPS is seed that is produced as a result of pollination. The seed is produced inside fruits that resemble small tomatoes on the potato vine. The major disadvantage of TPS is that it segregates for numerous traits because potato is highly heterozygous and each seed produced is potentially a new variety. When TPS is used for planting, harvested tubers will be variable in quality. TPS plants take longer to establish themselves and set tubers, resulting in lower yield compared to plants grown from tuber seed. Some growers in tropical areas use TPS because it is easy to ship and free of pathogens (Dwelle 2003). Love (1994) reported that botanical seed (TPS, not tubers or “seed potatoes”) can survive and germinate for periods of time in excess of seven years however, it is unlikely that plants from TPS would persist for the reasons stated above.

2.13. Conclusions: The Biology of Potato

The propagation of commercial potato varieties through cloning mitigates concerns about increased pest potential such as seed dispersal, survival outside of cultivation, or outcrossing. Potatoes are rarely grown from true potato seed because the offspring would show such extreme diversity they would not be useful as commercial potatoes. In addition, potatoes are not known to escape from commercial fields or show weediness potential. Wild potato varieties are rare in the United States and

geographically isolated from commercial production areas, further reducing concerns about cross-pollination with wild species.

An understanding of the biology of potatoes shows the extremely difficult challenges associated with simultaneously incorporating multiple traits through traditional breeding. Thus, potatoes benefit greatly by the use of Innate™ technologies which provide a superior alternative to breeding, through the addition of non-coding DNA, and result in new potato varieties that are not plant pests.

3. Method of Development: Description of Marker-Free DNA Transformation

3.1. Introduction

Simplot's Innate™ technologies comprise many aspects of plant biology all working together to produce traits of interest in transformed plants. Specifically, the potatoes were modified to reduce asparagine and black spot bruise. Along with the potato derived expression cassettes, are promoters and spacers, also from the potato genome, all creating the gene silencing system. The targeted genes are then combined with the backbone elements of pSIM1278, to create the vector that is inserted into *Agrobacterium.* Potato plants are transformed by *Agrobacterium,* resulting in the incorporation of the expression cassettes that result in the desired traits of interest. A part of this process is the selection of potatoes to receive the new technology, and in this case, we chose some of the most prominent varieties for processing chips and fries. The pSIM1278 acts by effectively reducing asparagine which leads to reduced acrylamide levels in cooked potatoes, as well as reducing black spot bruise incidence.

Description of the Transformation System
The C58-derived *Agrobacterium* strain AGL1 was developed by precisely deleting the transfer DNA of the hyper-virulent plasmid pTiBo542 (Lazo *et al.* 1991). Transformed plants were grown on media containing the antibiotic timentin, which prevents survival of *Agrobacterium,* thus selecting for plants that are free of *Agrobacterium.* At the conclusion of the selection process, all *Agrobacterium* has been removed, no antibiotics remain, and the result is that genomic DNA from the potato or sexually compatible species have been added to the new plants.

Transformation Method
Stock plants were maintained in magenta boxes with 40 ml half-strength M516 medium containing 3% sucrose and 2 g/l gelrite (propagation medium). Potato internode segments of four to six mm were cut from four-week old plants, infected with the *Agrobacterium* AGL1 strain carrying pSIM1278, and transferred to tissue culture media containing 3% sucrose and 6 g/l agar (co-cultivation medium). Infected explants were transferred, after two days, to M404 medium containing 3% sucrose, 6 g/l agar and 150 mg/l timentin to eliminate *Agrobacterium* (hormone-free medium). Details of the methods are described in Richael *et al.* (2008).

Although *Agrobacterium* is effective in cleaving at the Right Border (RB) site, it often failed to fully release the DNA insert from its plasmid vector by also cutting at the Left Border (LB) site (Gelvin 2003). Consequently, some infected plant cells received the DNA insert itself as well as additional vector backbone sequences containing the backbone marker gene for cytokinin. These cells started expressing the isopentenyltransferase (*ipt*) gene resulting in production of the plant hormone cytokinin, which commonly regulates growth and development processes in plants.

After one month, the infected explants were transferred to fresh medium lacking any synthetic hormones and incubated in a Percival growth chamber under a 16 hr photoperiod at 24^{o} C where they started to form shoots. Many shoots expressed the *ipt* gene and displayed a cytokinin overproduction phenotype; these shoots were discarded and not considered for further analyses. PCR genotyping demonstrated that about 0.3 to 1.5% of the remaining shoots contained at least part of the DNA insert while lacking the *ipt* gene. Details on *ipt*-based marker-free plant transformation were published by Richael *et al.* (2008).

The process of eliminating *Agrobacterium* started two days after explant infection. For this purpose, tissues were subjected to the antibiotic timentin (150 mg/l) until proven to be free of live *Agrobacterium*. Proof was obtained by incubating stem fragments of transformed events on nutrient broth-yeast extract (NBY medium) for 2 weeks at 28^{O}C (repeated twice). In accordance with 7 CFR Part 340, transformed plants were transported and planted in the field only when free of live *Agrobacterium*. The potato events were confirmed to be free of *Agrobacterium*-derived vector backbone DNA by the following three methods: 1) If plants had the negative selectable isopentenyl isomerase (*ipt*) marker gene in the vector backbone, they were discarded. Plants with *ipt* gene expression would have stunted phenotypes, abnormal leaves, or the inability to root due to the cytokinin overproduction caused by *ipt* expression; 2) Absence of the backbone DNA was confirmed with Southern blot hybridization; 3) PCR was used to confirm no fragments of the backbone DNA were present.

3.2. Parental Varieties

Russet Burbank. Luther Burbank developed this variety in the early 1870s. Plants are vigorous and continue vine growth throughout the season. Stems are thick, prominently angled and finely mottled. Leaflets are long to medium in width and light to medium green in color. The blossoms are few, white and not fertile. The cultivar is tolerant to common scab but is susceptible to *Fusarium* and *Verticillium* wilts, leafroll and net necrosis and virus Y. Plants require conditions of high and uniform soil moisture and controlled nitrogen fertility to produce tubers free from knobs, pointed ends and dumbbells. Jelly-end and sugar-end develop in tubers when plants are subjected to stress. The tubers produced are large brown-skinned and white-fleshed, display good long-term storage characteristics, and represent the standard for excellent baking and processing quality. The variety is sterile and widely grown in the Northwest and Midwest, especially for the production of french fries.

Ranger Russet. This full season variety was released in 1991. Ranger Russet is more resistant than Russet Burbank to *Verticillium* wilt, viruses X and Y, leafroll and net necrosis, and *Fusarium* dry rot. It is highly resistant to hollow heart. Plants are large and upright to spreading. Stems are thick, green that can be light brownish to light purple in full sun. Leaves are large, broad and medium green. Flowers are abundant and produce viable pollen. Buds are green with reddish-purple base and pedicel and moderate amount of short pubescence. Corolla is medium large, red-purple color and anthers are bright yellow. It produces high yields of good quality, high specific gravity tubers that are long and slightly flattened, and well suited for baking and processing into french fries. Tubers are susceptible to common scab and black spot bruise. Ranger Russet matures earlier than Russet Burbank and would be considered a medium-length storage variety. The variety is fertile and mainly grown in the Northwest, especially for the production of french fries.

Atlantic. Plants are moderately large, with thick, upright stems, and slightly swollen, sparsely pubescent nodes. Leaves are bright, medium green, smooth, and moderately pubescent with prominent wings, large asymmetrical primary leaflets and numerous secondary and tertiary leaflets. Flowers are profuse

with green, awl-shaped, pubescent calyx lobes, pale lavender corolla, orange anthers and abundant, viable pollen. The cultivar is tolerant to scab and *Verticillium* wilt, resistant to pinkeye, highly resistant to Race A of golden nematode, virus X, tuber net necrosis, and shows some resistance to black spot bruise. Tubers are susceptible to internal heat necrosis, particularly in sandy soils in warm, dry seasons. Hollow heart in the larger diameter tubers (diameter > 4 inches) can be serious in some growing areas. Tubers are oval to round with light to heavy scaly netted skin, moderately shallow eyes, and white flesh. Tuber dormancy is medium-long. With high yield potential, high specific gravity and uniform tuber size and shape, Atlantic is the standard variety for chipping from the field or from very short-term storage (Webb *et al.* 1978). The variety is fertile and mainly grown in the Northeast and Southeast, especially for the production of chips.

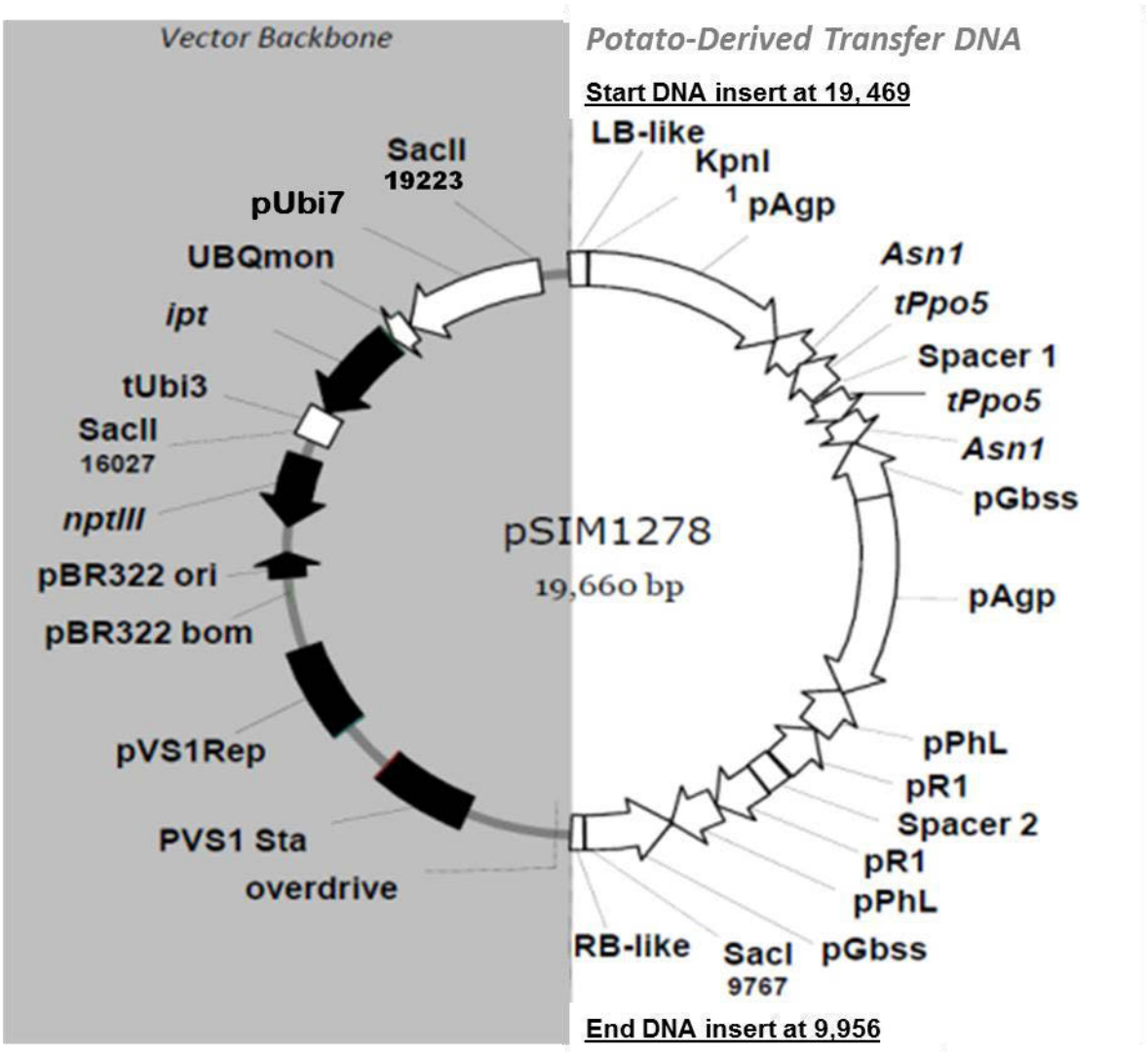
Variety G. Mid-season chipping variety with medium size tubers, round shape, shallow eyes, smooth slightly netted skin, white flesh, and high solids. It can be used as a fresh crop variety through mid-term storage. It generally has low defects but can display pressure bruise late in the storage season. It appears to be fairly resistant to PVY, is tolerant to common scab, and is confirmed to have resistance to the golden nematode *Globadera rostochiensis* Ro1. It has moderate flowering with white flowers which are both male and female fertile.

Variety H. Mid to late season chipping variety with large round to oval tubers, somewhat flattened shape, slightly indented eyes, smooth tan skin, pale yellow flesh, and has low to medium solids relative to other chipping varieties. It is generally used as a mid to late season storage variety. It is resistant to tuber early blight but susceptible to common scab. It has sparse white flowers and has no male fertility and very low female fertility.

3.3. Plasmid used for Transformation

The plasmid pSIM1278 is a binary transformation vector consisting of two parts: vector backbone and the DNA insert (**Figure 3**). The backbone section contains well-characterized bacterial origins of replication from plasmids pVS1 and pBR322, and the *nptIII* gene for bacterial resistance to kanamycin (see **Table 4** for details of the backbone of pSIM1278). An expression cassette comprising the *Agrobacterium ipt* gene preceded by the polyubiquitin (Ubi7) promoter (GenBank accession no. U26831.1) and followed by the Ubi3 terminator was introduced as a 2.6-kb SacII fragment into the vector backbone (Garbarino and Belknap 1994). The DNA insert consists of the genetic elements described in **Table 5**.

Figure 3. Vector pSIM1278



The vector backbone region, on the left, starts at position 9,957-bp and ends at 19,468-bp (= 9,512-bp). The backbone DNA consists mainly of bacterial DNA and is intended only to support maintenance of the DNA insert prior to plant transformation. The DNA insert region, including flanking Border sequences, is on the right from 19,469-bp to 19,660 and from 1-bp to 9,956, which is a total of 10,148-bp.

3.4. Backbone Portion of pSIM1278

The purpose of the backbone portion of pSIM1278 is to maintain the DNA insert in bacteria; it is not intended for transfer to plant cells. The various elements of the backbone are described in **Table 4**.

Table 4. Genetic elements of the pSIM1278 backbone

Genetic Element	Origin	Intended Function	Other effects on plant	Genbank Accession Number	Start-End Point in pSIM1278 (bp)	Reference
SacII restriction site	*S. tuberosum*	Site for connection of Ubi7 promoter with LB flanking sequence.	*None*	AJ272136.1	19,219-19,224	
Polyubiquitin promoter (**Ubi7**) including the coding sequence for a 76-amino-acid potato ubiquitin monomer (**UBQmon**)	*S. tuberosum* var. Ranger Russet	Drives expression of the *ipt* backbone marker gene	*None*	U26831.1	17,479-19,218	
Isopentenyl transferase (***ipt***) gene	*Agrobacterium tumefaciens*	condensation of AMP and isopentenylpyrophosphate to form isopentenyl-AMP, a cytokinin	*Cytokinin formation*	NC_002377.1	16,744-17,466	Smigocki and Owens 1988
Terminator of the ubiquitin-3 gene (**tUbi3**)	*S. tuberosum*	Terminate *ipt* gene transcription	*None*	GP755544.1	16,038-16,392	Garbarino and Belknap 1994
Neomycin phosphotransferase III (***nptIII***) gene	*E. coli*	Aminoglycoside phosphotransferase	*None*	FJ362602.1	15,048-15,842	Courvalin *et al.* 1977
Origin of replication for pBR322 (**pBR322 ori**)	*E. coli*	Start position for plasmid replication in bacterial cells	*None*	J01784.1	14,477-14,757	*
(**pBR322 bom**)	*E. coli*	pBR322 region for replication in *E. coli*	*None*	J01749.1	14,077-14,337	*
pVS1 replicon (**pVS1Rep**)	*Pseudomonas fluorescens* plasmid pVS1	pVS1 region for replication in *Agrobacterium*	*None*	AJ537514.1 (4,501-5,501)	12,667-13,667	*
pVS1 partitioning protein StaA (**PVS1 Sta**)	*Pseudomonas fluorescens* plasmid pVS1	pVS1 stability	*None*	AJ537514.1 (6,095-7,095)	11,074-12,074	*
overdrive	*Agrobacterium tumefaciens*	Enhances cleavage at the Right Border site	*None*	K00549.1 (103-132)	9,963-9,992	*

*: see: http://www.cambia.org/daisy/cambia/585.html - (General structure map of pCAMBIA vectors)

3.5. The Transfer DNA (DNA insert) of the Vector

The transfer DNA (DNA insert) consists of non-coding genetic elements described in **Table 5**. The DNA insert or a functional part thereof, is the only part of vector pSIM1278 that is present in selected transformed events.

Table 5. Genetic elements of the DNA insert of pSIM1278, from Left Border site to Right Border site

Genetic Element	*Origin*	Intended Function	Genbank Accession Number	Start-End Point in pSIM1278	Reference
1. Left Border (LB) site[1]	Synthetic	Site for secondary cleavage to release single-stranded DNA insert from pSIM1278	AY566555[2] (bases 1-25)	19,469 – 19,493	van Haaren et al. 1989
2. DNA flanking the LB sequence	*S. tuberosum* var. Ranger Russet	Supports secondary cleavage at LB	AY566555[2] (bases 26-187)	19,494 – 19,655	
3. KpnI restriction site	*S. tuberosum*	Site for connection of DNA insert with LB flanking sequence.	AF393847.1	19,656 –1	
4. Promoter for the ADP glucose pyrophosphorylase gene (pAgp), 1st copy	*S. tuberosum* var. Ranger Russet	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of Asn1 and Ppo5, especially in tubers	HM363752	2-2,261	
5. Fragment of the asparagine synthetase-1 (Asn1) gene (1st copy antisense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (9) double stranded RNA that triggers the degradation of Asn1 transcripts to impair asparagine formation	HM363759	2,262-2,666	Chawla et al. 2012[3]
6. 3'-untranslated sequence of the polyphenol oxidase-5 gene (Ppo5) (1st copy, in antisense orientation)	*S. verrucosum*	Generates with (8) double stranded RNA that triggers the degradation of Ppo5 transcripts to block black spot bruise development	HM363754	2,667-2,810	
7. Spacer-1	*S. tuberosum* var. Ranger Russet	Sequence between the 1st inverted repeats	HM363753	2,817-2,973	
8. 3'-untranslated sequence of the polyphenol oxidase-5 gene (Ppo5) (2nd copy, in sense orientation)	*S. verrucosum*	Generates (6) double stranded RNA that triggers the degradation of Ppo5 transcripts to block black spot bruise development	HM363754	2,974-3,117	
9. Fragment of the asparagine synthetase-1 (Asn1) gene (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (5) double stranded RNA that triggers the degradation of Asn1 transcripts to impair asparagine formation	HM363759	3,118-3,523	Chawla et al. 2012[3]
10. 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	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of Asn1 and Ppo5, especially in tubers	HM363755	3,530-4,215	
11. pAgp, 2nd copy	*S. tuberosum* var. Ranger Russet	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	HM363752	4,232-6,491	
12. Fragment of promoter for the potato phosphorylase-L (pPhL) gene (1st copy, in antisense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (16) double stranded RNA that triggers the degradation of PhL transcripts to limit the formation of reducing sugars through starch degradation	HM363758	6,492-7,000	
13. Fragment of promoter for the potato R1 gene (pR1) (1st copy, in antisense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (15) double stranded RNA that triggers the degradation of R1 transcripts to limit the formation of reducing sugars through starch degradation	HM363757	7,001-7,532	
14. Spacer-2	*S. tuberosum* var. Ranger Russet	Sequence between the 2nd inverted repeat	HM363756	7,539-7,796	

Table 5 continued. Genetic elements of the DNA insert of pSIM1278, from Left Border site to Right Border site

Genetic Element	Origin	Intended Function	Genbank Accession Number	Start-End Point in pSIM1278	Reference
15. Fragment of promoter for the potato R1 gene (pR1) (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (13) double stranded RNA that triggers the degradation of R1 transcripts to limit the formation of reducing sugars through starch degradation	HM363757	7,797-8,328	
16. Fragment of promoter for the potato phosphorylase-L (pPhL) gene (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (12) double stranded RNA containing this sequence will trigger the degradation of PhL transcript to limit the formation of reducing sugars through starch degradation	HM363758	8,329-8,837	
17. pGbss (2nd copy, convergent orientation relative to the 2nd copy of pAgp)	*S. tuberosum* var. Ranger Russet	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	HM363755	8,838-9,761	
18. SacI restriction site	*S. tuberosum*	Site for connection of DNA insert with RB flanking sequence.	AF143202	9,76 – 9,767	
19. DNA flanking the RB sequence	*S. tuberosum* var. Ranger Russet	Supports primary cleavage at RB-Like site	AY566555[2] (bases 231-391)	9,77 – 9,931	
20. Right Border (RB) sequence[1]	Synthetic	Site for primary cleavage to release single stranded DNA insert from pSIM1278	AY566555[2] (bases 392-416)	9,932 - 9,956	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]GenBank Accession AY566555 was revised to clarify the sources of DNA for the Border regions.
[3]ASN1 described as genetic elements 5 and 9 is referred to as StAst1 in Chawla *et al.* 2012.

3.6. Open Reading Frames Associated with the DNA Insert

Open reading frames (ORFs) are long sequences of DNA (a few hundred base pairs) that start with an ATG triplet and end with a stop codon. ORFs may encode part or all of a protein if flanked by appropriate regulatory sequences.

The full 10,148-bp DNA insert, from the cleavage site in the Left Border element to the cleavage site in the Right Border element contains fragments associated with an ORF of the *Asn1* gene and the trailer associated with an ORF of the *Ppo5* gene. These inverted repeats produce a pool of variably-sized RNAs that trigger gene silencing and are not translated into protein when transcribed.

3.7. Conclusions of Methods of Development: Description of Marker-Free DNA Transformation

A description of Innate™ technologies outlines the plant biological systems all working together to create the plants. These include trait identification, design of vectors, incorporation of vectors into *Agrobacterium*, recipient potato variety selection, transforming plants, and confirmation that the new potatoes contain the expected DNA inserts. The Innate™ methods allowed us to insert the desired traits into potato varieties using non-coding DNA into potato and develop new potato events that are not plant pests.

4. Characteristics of Transferred DNA and Gene Regulation

The potato events all produce potatoes that are silenced for specific genes. The silencing methods are part of Simplot's Innate™ technologies for reducing gene expression. This patented method provides the framework for reducing the expression of genes that ultimately reduce black spot bruise, asparagine, sugars, and acrylamide in potatoes. A brief introduction to gene silencing is given here, describing how similar methods are found in nature. In general, the inserted DNA contains silencing cassettes that, when expressed, generate variably-sized and unprocessed transcripts. These transcripts trigger the degradation of mRNAs that would normally code for an enzyme, like asparagine synthetase. This results in much reduced levels of the targeted "silenced" enzymes.

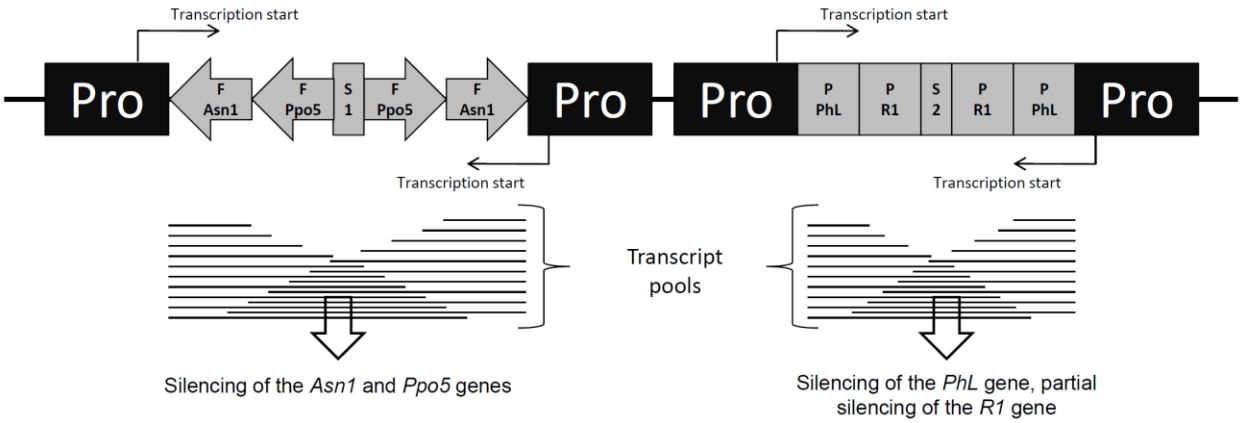
All potato events were analyzed by DNA gel blot analyses to determine the structure and copy number of the integrated DNA insert sequences and to confirm the absence of vector backbone sequences. These studies were carried out as parts of the characterization and biosafety assessment of the events. In addition, molecular characterization was used to determine the sequence of the junctions flanking the DNA insert and show stability of the inserted DNA. Sequencing information of the junctions provided the basis for developing event-specific PCR tests for all events.

4.1. Description and History of the Modification

Mechanism for Gene Silencing

The silencing approach applied here to down-regulate the transcript levels of several genes is illustrated in **Figure 4**. Two copies of a DNA segment comprising fragments of four targeted genes are inserted, as an inverted repeat, between two convergent promoters that are predominantly active in tubers. Plants containing the silencing cassette produce a diverse and unpolyadenylated array of RNA molecules in tubers that dynamically and vigorously down-regulate the intended target genes. The size of the RNA molecules is generally smaller than the distance between the two promoters according to Yan *et al.* 2006.

Figure 4. Silencing Approach Using the DNA Insert of pSIM1278



(Pro = functionally-active promoter, F = gene fragment, S1 = spacer 1, P = non-functional promoter fragment, S2 = spacer 2)

The expression of an inverted repeat is a regulatory system that has evolved naturally as a consequence of the duplication of genes and regulatory elements. One example relates to a 27-kb region of the

soybean genome containing two perfectly repeated and inverted clusters of three chalcone synthase (*Chs*) genes (Tuteja *et al.* 2009). This results in the silencing of all *Chs* gene family members through the formation of double-stranded RNA. In this example, the double-stranded RNA initiates gene silencing, and is an example of naturally occurring inhibition of a metabolic pathway, which functions specifically in one tissue while allowing expression in other parts of the plant.

Expression of Neighboring Genes

Previous studies on convergent transcription have shown that transcripts are of variable size but smaller than the distance between the two promoters, both in yeast (Prescott and Proudfoot 2002) and plants (Yan *et al.* 2006). In a study on convergent transcription-based silencing of the β-glucuronidase (*gus)* gene, no expression of neighboring genes was found (Yan *et al.* 2006). The RNA gel blot analyses presented in this petition (**Appendix 5. Efficacy and Tissue-Specificity of Gene Silencing**) demonstrated the absence of bands or smears indicative of read-through transcripts that hybridized with a probe derived from the entire *Asn1*/*Ppo5* inverted repeat (see, for instance, **Figure 2** of **Appendix 5. Efficacy and Tissue-Specificity of Gene Silencing**).

Although the possibility of rare read-through transcripts cannot be excluded, it is unlikely that such transcripts would interfere with the expression of neighboring genes because single stranded transcripts are ineffective in triggering gene silencing (Yan *et al.* 2006). Furthermore, studies on the expression of an antibiotic selectable marker gene next to a convergent transcription-based silencing construct demonstrated that this antibiotic gene was not affected by expression of the silencing construct (Yan *et al.* 2006). In the unlikely event that read-through occurred, and lowered the expression of an adjacent gene in any of the 10 events, it would have been evident through change in plant phenotype. After extensive evaluations from multiple field trials over three years, no unexpected agronomic, phenotypic, or biochemical differences were observed with these 10 events when compared with control varieties.

4.2. Verification of Insert Integrity and Number of Inserted Sequences

A diagram of the structure of inserts is shown in **Figure 5** (see **Abbreviations** for most keys; AGP = pAgp, GBS = pGbss). Details on characterization of the inserted DNA are provided in **Appendix 1. Characterization of Inserted DNA**. Two events (J78 and G11) contain a single truncated copy of the DNA insert with a functionally-active *Asn1*/*Ppo5* gene silencing cassette (and part of the R1/PhL promoter silencing cassette that is not functionally active).

The comprehensive molecular analyses confirmed anticipated similarities between the events of this petition and T-DNA integration events described elsewhere:

(1) Six events (F10, F37, E12, E24, J78, and G11) contain 1 copy (whereby "copy" implies the presence of at least an *Asn1*/*Ppo5* gene silencing cassette), three events contain 2 copies (J3, J55, and H50), and the remaining event H37 contains 1 copy with 3 additional fragments. This finding indicated that the number of copies of the DNA insert is similar to that reported elsewhere for T-DNAs in potato (Wolters *et al.* 1998) and other crops such as tobacco (De Neve *et al.* 1997; De Buck *et al.* 1999), *Arabidopsis* (De Buck *et al.* 2009), rice (Sallaud *et al.* 2003; Yang *et al.* 2005), wheat (Cheng *et al.* 1997), and barley (Bartlett *et al.* 2008). Although high copy numbers have been linked to post-transcriptional gene silencing (Lechtenberg *et al.* 2003), we found no differences in the extent and persistence of silencing activities between higher-copy events and the events with only one copy.

(2) Event J55 contained two linked DNA inserts positioned as an inverted repeat. Lechtenberg *et al.* (2003) showed with bacterial T-DNA that the presence of a second gene copy either in tandem

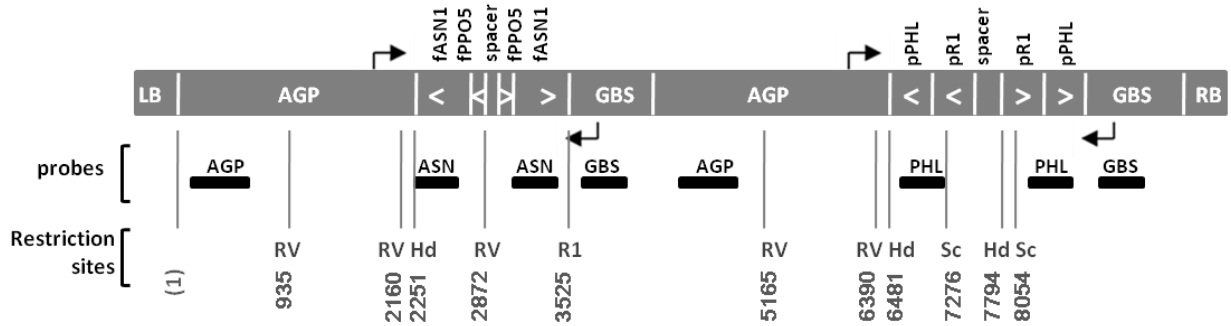
or an inverted arrangement did not result in silencing. Thus, it's likely that the inverted linked DNA insert copies in J55 would not contribute to silencing and therefore, the silencing of targeted genes functions as intended based on the inverted repeats positioned between convergent promoters.

(3) Inserts are occasionally flanked by short DNA sequences that are derived from the plant genome or the DNA insert. These insertions appear to be part of the integration process and occur at rather high frequencies (Windels *et al*. 2003). An example of an event with such sequences includes the 49-bp sequence between the two DNA inserts of J55. A blast search of this short DNA sequence using GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome) partially matched known sequences from *S. tuberosum,* confirming that the origin was most likely from either the plant genome or the DNA insert.

(4) Most transferred DNA inserts are shorter than the full distance between the Left and Right Border sequences, as shown by short deletions near the borders. Such deletions are also associated with T-DNA integration and hypothesized to result from double-strand break repair (Gheysen *et al.* 1991). Short deletions that did not impair the functional activity of any of the two silencing cassettes were found in, for instance, events F10 (a 38-bp deletion at the Right Border) and F37 (a 12-bp deletion at the Left Border). Large deletions at the Right Border side that resulted in loss of the PhL/R1 promoter silencing cassette occurred in events J78 and G11.

Figure 5. Diagram of DNA Inserts

F10 Insert:



F37 Insert:

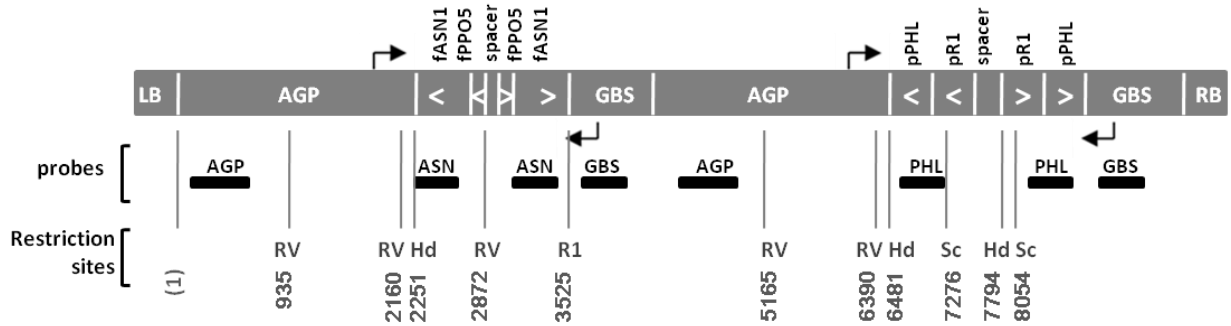
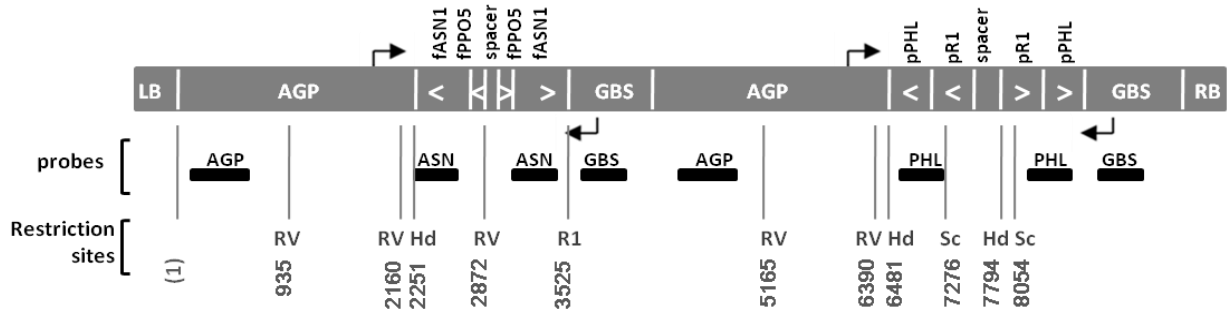
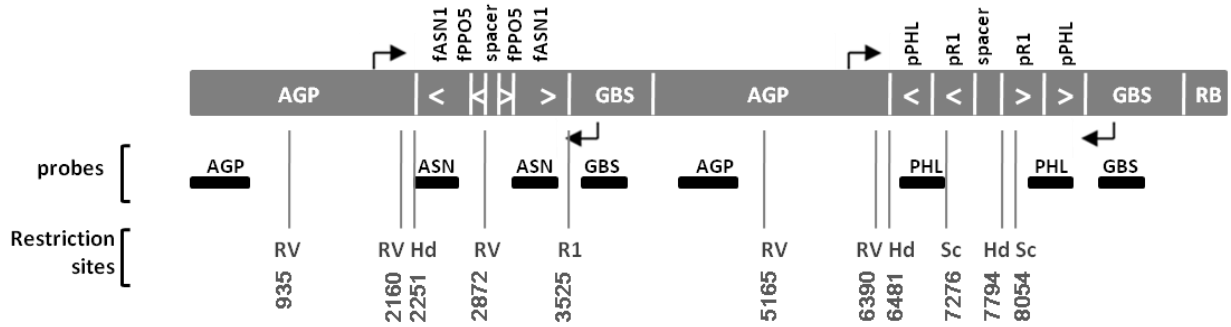


Figure 5 (Continued). Diagram of DNA Inserts

E12 Insert:



E24 Insert:



J3 Insert:

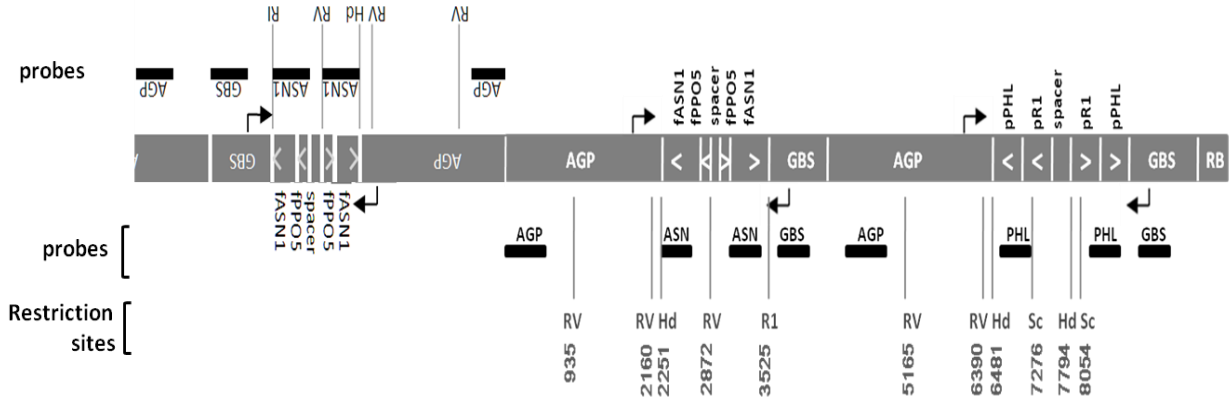
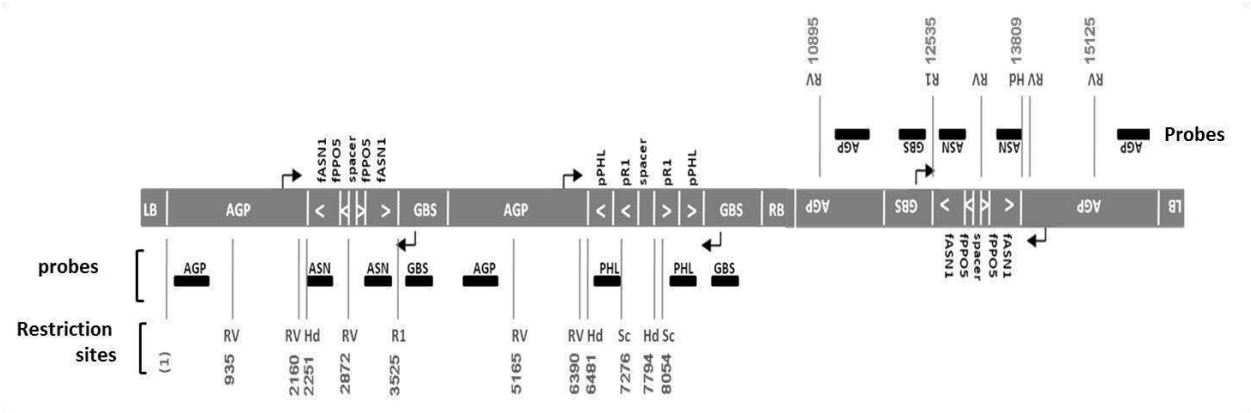
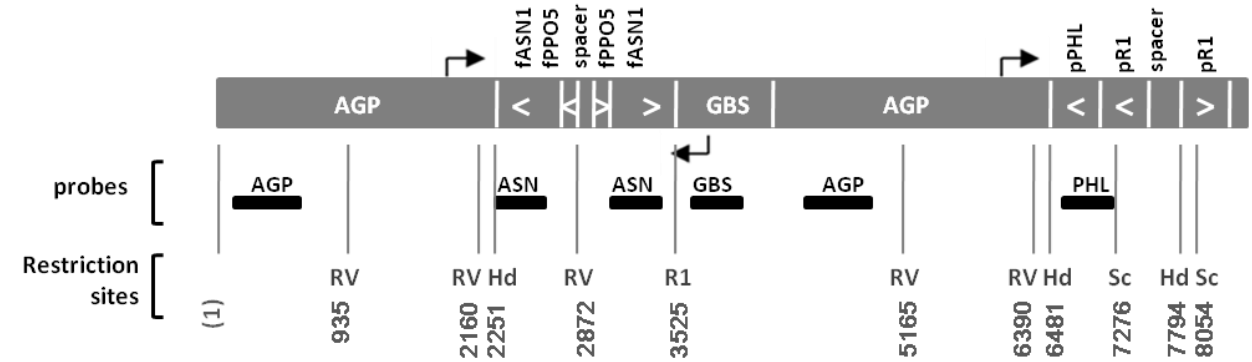


Figure 5 (Continued). Diagram of DNA Inserts

J55 Insert:



J78 Insert:



G11 Insert:

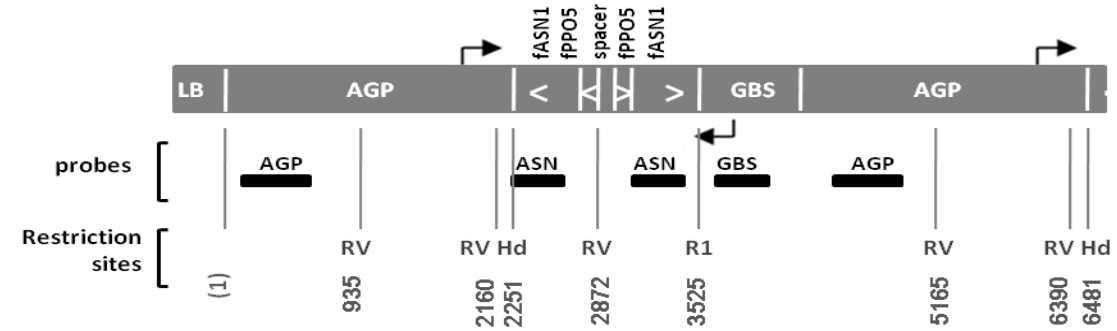


Figure 5 (Continued). Diagram of DNA Inserts

H37 Insert:

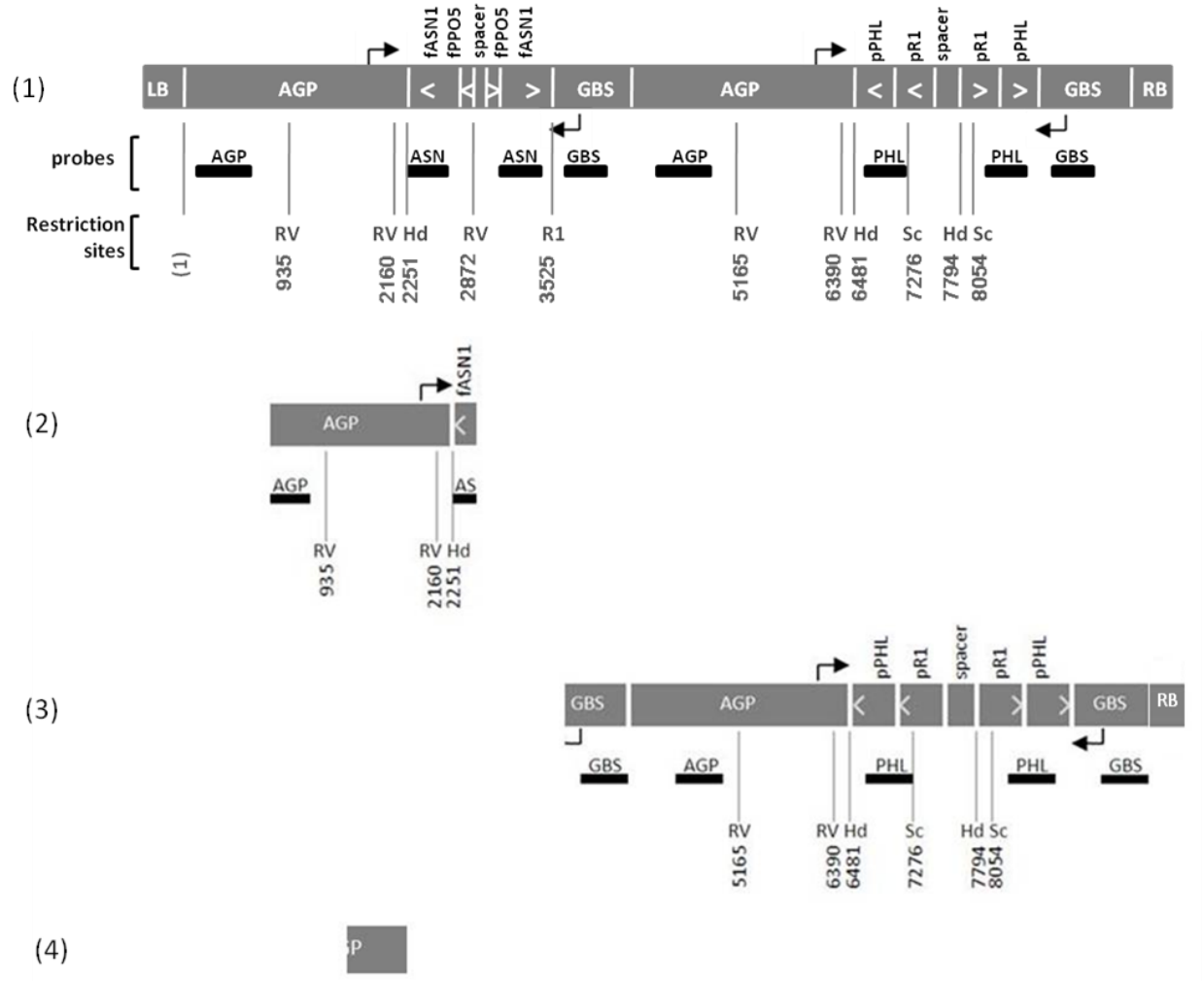
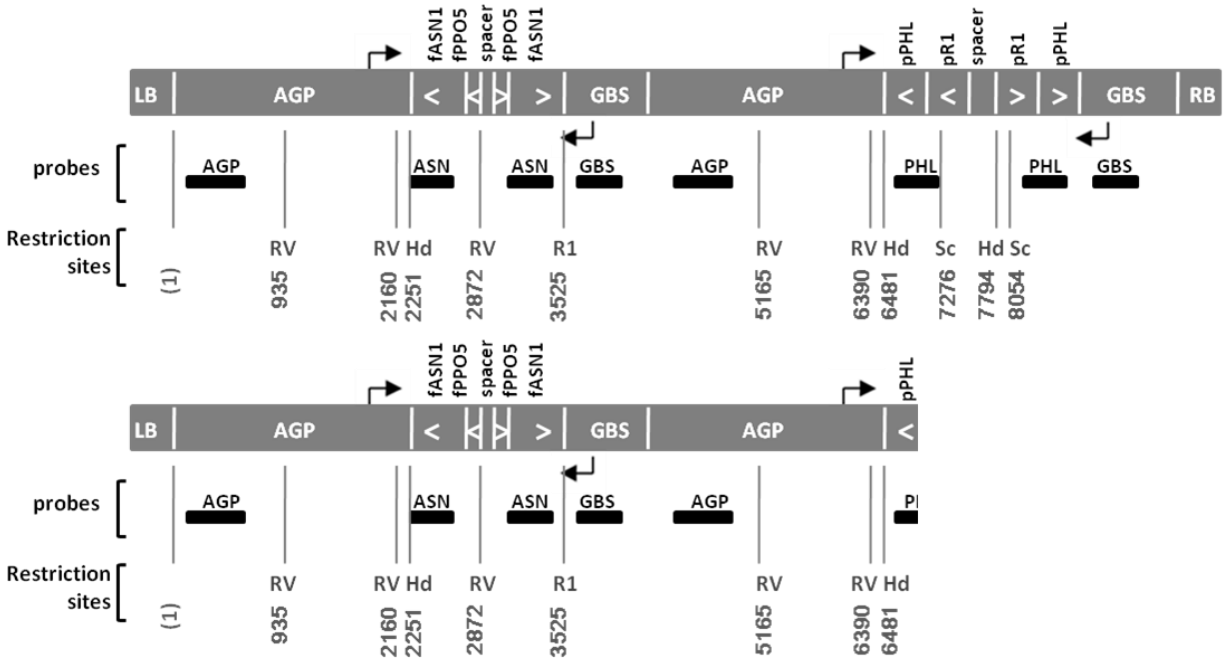


Diagram (4) is a small fragment containing part of the AGP promoter.

Figure 5 (Continued). Diagram of DNA Inserts

H50 Insert:



4.3. Selection of Backbone-free Plants

Agrobacterium-mediated transformation often results in transfer of "backbone" sequences that are vector DNA in addition to the intended sequences positioned between the Left and Right Borders of the plant derived DNA insert. The frequency of transfer of backbone DNA was estimated at 75% for tobacco (Kononov *et al.* 1997) and 47% to 67% for *Arabidopsis* (Oltmanns *et al.* 2010).

In contrast, all the events in this submission were confirmed to be free of vector backbone DNA (**Appendix 2. Evidence for the Absence of Vector Backbone DNA**). This finding confirms previously published work on Simplot's cytokinin vectors (Richael *et al.* 2008).

4.4. Flanking Regions of Inserted Sequences

Extensive research was conducted to characterize the inserted DNA for all events (**Appendix 1. Characterization of Inserted DNA**). In addition, at least one junction was sequenced for all events to develop event-specific PCR methods.

Among the evidence supporting safety relative to the insertion site are the following: (1) most insertions do not disrupt gene expression because DNA integrates at random when no selection is used during transformation (Kim *et al.* 2007) and only approximately 5.5% of the potato genome is occupied by genes (Zhu *et al.* 2008); (2) insertions that do disrupt gene functions are almost always recessive (Hagio *et al.* 2002; Chiou *et al.* 2006; Daxinger *et al.* 2008), which means that change to a single chromosome would be unlikely to result in an observable phenotype (in contrast to most other crops, transformed potatoes are not self-fertilized (backcrossed) to develop events that are homozygous for the insert); and

(3) if an unusual and undesirable phenotype occurred, that event would be selected only if equivalent or superior to the untransformed potato variety.

4.5. Stability of Inserted DNA

Bacterial T-DNAs are not always stable after insertion into a plant. The estimated instability rate of between 0.5-5.9×10^{-4}, is associated with meiosis (Müller *et al.* 1987; Conner *et al.* 1998). Because potato is a vegetatively propagated crop and reproduction does not involve meiosis, DNA insertions were expected to be stable. Nevertheless, the stability of integrated DNA inserts was confirmed both by DNA gel blot hybridization and by assaying G0, G1, G2, and G3 tubers for polyphenol oxidase activity (**Appendix 3. Evidence for Stability of the Inserted DNA**).

It should be mentioned here that one event that is not considered in this submission for nonregulated status, designated as G09, was found to be unstable. About 2% of the G2 tubers of this event had partially lost black spot bruise tolerance (**Appendix 3. Evidence for Stability of the Inserted DNA**). Such instability may have been associated with the unusually complex DNA insert organization of G09 (7 copies of at least part of the DNA insert). While not certain that the large copy number was the cause of instability, it would be recommended to select events with the minimum number of copies needed to express the desired traits. For example, it would be desirable to select future events with relatively simple DNA insertions (2 copies or less).

Although instability is undesired and may result in a phenotypic reversion back to wild-type where tubers lose the incorporated traits (black spot bruise tolerance, reduced acrylamide-forming potential, and, in some cases, reduced formation of fructose and glucose), it would not trigger any biosafety issues. To date, all assessments have been limited to G0, G1, G2, and G3 material. Continued testing will monitor phenotypic stability of the new traits.

4.6. Event-Specific PCR

As part of our stewardship and identity preservation plan as well as for internal event detection, event-specific PCR was developed for all the events submitted for nonregulated status. The methods developed will be used to monitor plants and tubers in field and storage as part of quality management programs (**Appendix 4. Junction Analysis and Event-Specific Detection**).

4.7. Conclusions: Characteristics of transferred DNA and Gene Regulation

We found the 10 events to contain at least 1 full or partial copy of the DNA insert from pSIM1278 and, in the case of H37, three additional fragments of pSIM1278. Although the inserts were not always full-length, therefore, each event contained at least one functional *Asn1*/*Ppo5* gene silencing cassette. This means there are three main differences between these events and their untransformed counterparts: 1) Innate™ tubers contained less asparagine, 2) more glutamine and 3) at least some degree of black spot bruise tolerance. The one exception being that conventional Atlantic already displays some black spot bruise tolerance. All of the events were free of detectable vector backbone DNA. Reduced asparagine contributes to significant reductions in acrylamide after heating, in all events, even if the insert contained only the *Asn1/Ppo5* silencing cassette and lacked the R1/PhL silencing cassette that would contribute to lower reducing sugars.

All insertions represent simplex insertions into tetraploid loci. This heterozygote genotype is commonly referred to as AAAa, whereby "a" represents an allele carrying the insertion, and "A" stands for lack of the insertion. Simplex insertions are unlikely to cause expression of recessive phenotypes, which usually require homozygote insertions (knock-out of all "A" dominant alleles).

It is possible but not likely that a promoter element positioned within an insertion could function as a long-distance enhancer or promoter for a neighboring open reading frame. Even if the expression of open reading frames adjacent to insert promoters had occurred, it would be rare that this would result in a functional and unwanted protein. If an insertion interrupted the expression of an essential gene, the interruption would be expected to disrupt the growth of the plant or change the composition of the plant tissues. The combined tests for molecular structure show that the potato events have resulted from the addition of genetic material from a donor organism where the material is well characterized and contains only non-coding regulatory regions of DNA.

5. Expression of the Gene Products

The DNA insert used to transform these ten potato events contains two expression cassettes designed to lower the transcript levels of endogenous genes. The first cassette contains two fragments of the asparagine synthetase-1 (*Asn1*) gene (404-bp) and the polyphenol oxidase-5 (*Ppo5*) gene (143-bp), inserted as inverted repeats between the convergent-oriented promoters of the ADP glucose pyrophosphorylase (*Agp*) and granule-bound starch synthase (*Gbss*) genes. The second cassette contains fragments of the promoters of the starch associated genes *R1* (531-bp) and phosphorylase-L (*PhL*) (508-bp), operably linked to the same regulatory elements used for the first cassette.

Transcript levels for the *Asn1* and *Ppo5* genes were reduced in the tubers, stolons, and, in some cases, other tissues of all potato events as expected (**Appendix 5. Efficacy and Tissue-Specificity of Gene Silencing**). It was also expected that transcript levels for the *PhL* and/or *R1* genes would be reduced in tubers, stolons, and, in some cases, other tissues of the potato events F10, F37, E12, E24, J3, J55, H37 and H50.

It is possible that these intended changes could trigger unintended effects because free asparagine and glutamine are signals of organic nitrogen status that regulates gene expression (Gutiérrez *et al.* 2008), while glucose stimulates the formation of abscisic acid and auxin while suppressing cytokinin production (Ramon *et al.* 2008). However, if unintended changes did occur, studies described in this petition demonstrated that they have no consistent impact on plant agronomics or tuber composition.

Previous studies have shown that *Ppo* gene silencing lowers the amount of protein to levels that are undetectable by Western blot analysis (Llorente *et al.* 2011). Similarly, silencing of the *R1* gene diminished accumulation of a ~160kDa protein that is at least partially bound to starch granules (Lorberth *et al.* 1998).

The inserted DNA in all 10 events included in this petition functions by silencing the genes involved with production of asparagine, polyphenol oxidase, and reducing sugars. By design, the events contain only non-coding regulatory sequences. Multiple studies included in this petition show the material to be well characterized and not associated with plant pest traits.

6. Efficacy and Tissue-Specificity of Gene Silencing

The purpose of these studies was to show the effectiveness of gene silencing based on RNA expression as measured through Northern blot analyses. Gene silencing methods were employed to lower the activity of the Asn1, Ppo5, PhL, and R1 native proteins. Transcript levels rather than protein amounts were evaluated to link new phenotypic traits to changes at the molecular level. For this purpose, RNA was isolated from various tissues of the events and their untransformed counterparts and used to carry out Northern blot analyses (see **Appendix 5. Efficacy and Tissue-Specificity of Gene Silencing**, with a summary in **Table 1**). Each letter (A, P, L, R) in **Table 6** indicates that silencing was confirmed, although the amount of silencing varied depending on the gene and tissue.

Innate™ technologies have given us the ability to selectively regulate genes in specific tissues rather than the entire plant. For example, strong silencing of the *Asn1* gene involved in ASN (asparagine) formation in potato tubers was desired, yet such changes in leaves and stems might adversely affect growth. The promoters used to drive gene silencing, the Agp promoter and the Gbss promoter, have been well characterized and are known to be highly active in tubers and stolons and less active in photosynthetically-active tissues and roots (Nakata *et al.* 1994; Visser *et al.* 1991). Therefore, silencing was predicted to be stronger in tubers and stolons, and less pronounced in leaves, stems, roots, and flowers, as shown in **Table 6**.

Table 6. Summary of Down-Regulated Genes in Different Tissues

Event	Tubers[1]	Stolons[1]	Roots[1]	Stems[1]	Leaves[1]	Flowers[1, 2]
F10	A P R	A L R	A P R	P	A	A
F37	A P R	A L R	A P R	A P		A R
E12	A P L R	A L R	A P	P		A
E24	A P L R	A P L R		A P		A
J3	A P L R	A R	A	A P		A
J55	A P L R	A L R	A	P		A
J78	A P	A		P		A
G11	A P	A P		R	R	A
H37[3]	A P L R	L R	P	P	R	
H50[3]	A P L R	A P L R	A P R	A P	R	

[1]A = *Asn1*, P = *Ppo*5, L = *PhL*, R = *R1*. Letters in table indicate down-regulated gene expression by tissue.

[2]The partially down-regulated *Asn1* gene expression might alter the amino acid composition of the flowers. Such effects will be limited to a reduction in ASN and an increase in GLN. Since ASN and GLN are similar non-essential amino acids, changes in the levels of these compounds is not expected to affect the quality of petal, nectar, and pollen as feed for insects or other organisms.

[3]Events derived from variety H do not produce flowers.

The targeted four genes (*Asn1, Ppo5, PhL, and R1*) are partially silenced in tubers of most events, except for J78 and G11 which do not contain the cassette for silencing the *PhL/R1* genes (**Table 6**). In addition, the *PhL* gene did not appear down-regulated in tubers of the Ranger Russet events, F10 or F37. For some events, E24, and H50, all four genes were also down-regulated in stolons. The two events J78 and G11, which contain a truncated DNA insert with a functionally active *Asn1*/*Ppo5* silencing cassette, produced tubers that contained reduced transcript levels for the *Asn1* and *Ppo5* genes only, since they did not contain the *PhL/R1* silencing cassette. Overall, more down-regulation of the four targeted genes occurred in tubers and stolons than in the roots, stems, leaves, or flowers (**Table 6**).

Event G11, which does not contain the PhL/R1 promoter silencing cassette, displayed reduced R1 transcript levels in leaves and stems. This unexpected change may be a consequence of DNA integration or somaclonal variation (Skirvin *et al*. 1993).

The expression studies showed that silencing was most effective in tubers and stolons, as predicted with the chosen promoters, Agp and Gbss. In addition, silencing was predictable based on the molecular characterization that showed all events contained the *Asn1* and *Ppo5* genes, but two events, J78 and G11 events, did not contain the *R1* and *PhL* genes, and therefore did not show silencing of those genes in tubers.

Considering the potato events in this study, expression of the targeted genes would provide evidence that the desired phenotype with low asparagine, reduced black spot, and lowered reducing sugars had been achieved. It could provide information relevant to the plant pest risk assessment, for example, all Innate™ potatoes in the study had reduced or similar expression to the controls. Thus, the altered expression is unlikely to affect weediness or other plant pest characteristics. Also, with these events, the expression studies pertain to endogenous proteins rather than new proteins that could require a safety evaluation, such as those associated with herbicide tolerance or insect resistance.

Additional evidence for the safety of these potato events compared to the untransformed controls is illustrated in the agronomy and composition studies. It can therefore be concluded that these modified expression levels do not impact plant agronomics or influence tuber composition. Thus, the expression studies provide additional evidence that the Innate™ potatoes are not plant pests and the integrated genetic material contains only non-coding regulatory regions that are well characterized, and are as safe as untransformed potatoes.

7. Agronomic Performance, Disease, and Efficacy of Traits

The purpose of the agronomic trials was to confirm that the Innate™ potatoes had equivalent phenotypes compared with their untransformed controls when grown at multiple locations representing the major areas for potato production in the US. Observations throughout the growing season allowed for a thorough assessment of growth, disease and pest susceptibility, and measurements of tuber yield and quality. The field assessments aided in the selection of the events that contained the intentionally incorporated new traits and maintained all benefits of the conventional parent varieties. These assessments also made it possible to ensure that the transformations had not resulted in the introduction of unintended effects associated with weediness or pest-like characteristics.

After completing the agronomic trials, potato tubers were tested for evidence of the efficacy of the traits, including an assessment for black spot bruise and reducing sugars. In addition, after processing into french fries or potato chips, the potatoes were tested for acrylamide. A brief summary of the acrylamide results will be presented here with the supporting details available in **Appendix 9. Compositional Analyses**.

7.1. Agronomy and Phenotypic Evaluation

This summary includes agronomic evaluations of 10 events, representing 5 varieties, at geographically distinct sites that represent most of the main production areas for potatoes destined for fry and chip production in the USA (**Appendix 6. Field Performance and Tuber Evaluations**). All agronomic trials were conducted under Biotechnology Quality Management System (BQMS) standards and USDA-APHIS compliance (For a complete listing of trials, see **Table 13** in **Section 14. Record of Field Test Reports**). Planting was carried out mechanically to facilitate subsequent harvests and to ensure that Innate™ potatoes were kept separate from unmodified material. The agronomic evaluations relate to both field observations and tuber assessments, both at harvest and during storage. **Table 7** shows which characteristics were evaluated. Some differences in data collected reflect differences in product use in processing, *e.g.*, Ranger Russet and Russet Burbank used for french fries and Atlantic, G, and H are used in the potato chip industry.

Table 7. Summary of Agronomic Observations

Characteristic	Number of Site Years[1]				
	Ranger Russet	Russet Burbank	Atlantic	G	H
Early Emergence	4	4	13	3	3
Final Emergence	13	4	4	3	3
Stems Per Plant	4	4	7	NA	NA
Plant Vigor	8-11	11	7-8	6	5
Foliage Color	8-11	11	14-15	6	5
Leaflet Size	8-11	11	14-15	6	5
Leaflet Curl	8-11	11	14-15	6	5
Senescence	3	3	6	NA	NA
Vine Size	NA	NA	NA	4	4
Vine Maturity Rating	7	7	9-10	5	5
Flower Color	2-4	3-4	7	NA	NA
Total Yield	11	11	14	3	3
% 4-6 oz.	9-10	8-10	NA	NA	NA
% 6-10 oz.	9-10	8-10	NA	NA	NA
% 10-14 oz.	9-10	8-10	NA	NA	NA
% >14 oz.	9-10	8-10	NA	NA	NA
Specific Gravity	9-10	8-10	14	3	3
High Sugar	9-10	8-10	NA	NA	NA
Sugar Ends	9-10	8-10	NA	NA	NA
Total Internal Defects	9-10	4	14	3	3
% U.S. #1	NA	NA	14	3	3
% Grade B	NA	NA	14	3	3
% Grade A	NA	NA	14	3	3
% Oversize	NA	NA	14	3	3
% Pickouts	NA	NA	14	3	3
Insect Stressors	3-5	5	7	1	1
Disease Stressors	3-5	5	7	1	1
Abiotic Stressors	3-5	5	7	1	1
Incidental Stressors	8	8	9	6	6

NA = characteristic not evaluated

[1] Total number of sites in 2009, 2010, and 2011 for each characteristic. Ranges indicate that not all lines of each variety were grown at every site.

The agronomic and ecological data that are presented in **Appendix 6** (**Field Performance and Tuber Evaluations**) show that over multi-year and multi-site field trials no variety specific differences leading to increased weed or pest potential were present. The studies were conducted with as much scientific rigor as possible to ensure product and trait safety. Statistical methods were employed to test for differences between the Innate™ varieties and appropriate controls. We used a step-wise approach to interpret any differences that were observed (both statistically significant and non-statistically

different). For each comparison between a test line (event) and the appropriate conventional control, the following logic was employed to assess the results. First, when p-values were available, statistical significance was determined for each comparison. If the p-value indicated no statistical significance, it was unlikely that there was a difference that would be biologically meaningful or indicative of increased pest potential. Next, if the p-value indicated statistical significance or the data did not support calculation of a p-value, the mean value of the test line was compared to the combined control range, which represents the minimum and maximum mean values of all conventional lines included in the experiments. If the value for the test line was within the combined control range, it was concluded that the test line exhibited characteristics within the natural variation of potatoes and that the difference was unlikely to be biologically meaningful or indicative of increased pest potential. If the value of the test line was outside the combined control range, further consideration was given to the difference in the context of agronomic impact and increased pest potential.

The combined control range for the agronomic characteristics used a total of 319 mean values from conventional Ranger, Burbank, Atlantic, G, and H varieties grown as controls in the field trials. For the yield and grading data for the Burbank and Ranger lines, the combined control range used a total of 170 mean values of the Burbank and Ranger conventional controls. For the yield and grading data for the Atlantic, G, and H lines, a total of 160 mean values of Atlantic, G, and H conventional controls were used to determine the combined control range.

Although some significant agronomic differences were observed between events and controls, most fell within the combined control range of the conventional varieties. For example, some differences in yield and tuber size were observed in the events, and these attributes will be investigated further in commercial trials. Thus, we concluded that no biologically meaningful differences that would contribute to increased weediness or pest potential were observed for any of the agronomic characteristics, yield and grading characteristics, or ecological interactions (namely plant-insect interactions, plant-disease interactions, and plant interactions with abiotic stressors) of the events compared to their untransformed controls **(Appendix 6. Field Performance and Tuber Evaluations**). There were no differences in phenotype and tuber composition (**Appendix 9. Compositional Analyses**) or disease susceptibility (**Appendix 8. Disease Susceptibility**). It can be concluded that the events are not different from their untransformed controls in terms of agronomy, tuber yield, and tuber composition. Therefore, the transformation of potato with pSIM1278 does not introduce characteristics that will encourage or enhance weediness or pest potential.

7.2. Disease susceptibility and polyphenol oxidase

Polyphenol oxidase enzymes (Ppo) are found in most organisms including animals, plants, fungi and bacteria. Although much is known about the molecular biology of Ppo and its role in enzymatic browning, little is understood about the function of Ppo-mediated browning in plant physiology. Some have hypothesized that Ppos represent part of the plant's defense response against insects and pathogens. Considering that the present submission involves silencing of Ppo in tubers, we reviewed relevant literature and conducted studies relating to the disease response of the events.

Ppos are copper metalloenzymes which oxidize mono- and o-diphenols to o-diquinones by utilizing molecular oxygen (Thipyapong *et al*. 2004). Typically, Ppo activity is latent until the enzyme is released by disruption of the cell structure through forces like wounding and senescence. When cell membranes are damaged, Ppo enzyme is released and reacts along with oxygen molecules to produce quinones

(Thipyapong *et al.* 2004). The production of black and brown quinones is responsible for much of the interest in PPO in the post-harvest physiology of many fruit and vegetable crops.

It is common for multiple homologues or alleles to exist within species, each responsible for expression in different plant tissues. In potato, 6 genes encoding Ppo have been identified, tomato (*Lycopersicon esculentum*) possesses 7 PPO genes, and banana (*Musa acuminata*) is known to contain a Ppo family of at least 4 genes (Thipyapong *et al.* 2007; Mayer 2006). A number of researchers evaluated induced Ppo activity and response mechanisms to biotic stressors, in particular pests and pathogens (Steffens *et al.* 1994). Studies with biotech tomatoes found a positive correlation between high levels of Ppo in leaf tissue and increased resistance to pathogens and insect pests. In one study, three lines with higher Ppo expression were tested against the bacterial pathogen *Pseudomonas syringae* pv. *Tomato*, the causal agent of bacterial speck in tomato (Li and Steffens 2002). These lines showed enhanced suppression of disease symptoms and exhibited 15-fold fewer lesions per leaf area than controls. Although these results indicated a protective effect of Ppo, the mechanism for disease resistance remains unknown (Li and Steffens 2002).

In potato, impacts sustained during harvest and postharvest activities induce the release of Ppo from cell plastids, facilitating oxidation of phenols to quinones, and resulting in negative effects on quality and recovery in processing french fries and chips, as well as the marketability of fresh potatoes. A family of 6 genes encoding Ppo exists in potato, with one gene (*Ppo5*) being tuber-specific and the remaining 5 genes responsible for Ppo expression in other tissues. The tuber-specific *Ppo5* gene was down-regulated, resulting in reduced susceptibility to black spot bruise, as shown in the current petition for nonregulated status.

Researchers found that by down-regulating Ppo activity in potato *e.g.*, see Hakimi *et al*. 2006, disease symptoms of *P. infestans* were reduced, apparently corresponding to an increase in the plant's resistance to late blight. The authors proposed that tubers may also display enhanced disease resistance against certain other fungal pathogens that infect potato tubers, including *Rhizoctonia* (black scurf), *Fusarium* (dry rot), *Spongospora* (powdery scab) and *Alternaria* (early blight).

Enzymatic browning is an important reaction that occurs in the fruit of the apple (*Malus domestica*). In a study conducted by Valentines *et al.* (2005), the roles of enzymatic browning and lignification (the chemical strengthening of cell walls in response to pathogenic infection) as resistance mechanisms against *Penicillium expansum* were investigated in Golden Delicious apples. A significant increase in decay was observed following the treatment of peeled apples with a Ppo substrate which had induced higher Ppo activity levels. The Ppo enzyme, and particularly the browning process induced by treatment with Ppo substrate, may have contributed to increase decay and indicates that an overexpression of Ppo may lead to higher level of sensitivity towards the pathogen (Valentines *et al.* 2005).

Some researchers have proposed that enhanced Ppo may increase resistance to disease, while others claim that reduced Ppo could also increase resistance. Considering that some evidence exists for a relationship between Ppo and diseases, we chose to test the events for several important potato diseases and conclude that there is no impact on disease resistance or susceptibility with regards to the *Ppo5* gene as demonstrated in the remainder of this section.

7.3. Disease Incidence: Storage Studies

Throughout the 2009 cold storage period, pink and soft rot diseases were occasionally observed on both untransformed and Innate™ potatoes from the Canyon County, ID, and Bingham County, ID, field trial sites. In addition, *Pythium* leak disease was observed on some untransformed and Innate™ potatoes from Adams County, WA, and Bingham County, ID, for the 2010 cold storage period. These observed disease incidences were attributed to environmental soil conditions that favored infection of tubers and the relatively high presence of disease within the specific field trial sites.

7.4. Disease Incidence: Late Blight and Bacterial Soft Rot

Disease incidence was evaluated by intentionally infecting the events and their untransformed controls with the causal agents of late blight (*Phytophthora infestans*) and soft rot (*Erwinia carotovora*) and evaluating disease progression. Details regarding the following tests are included in **Appendix 8. Disease Susceptibility**.

Late Blight Testing with Tubers. Results of studies from the 2009 field trials showed an increase in late blight symptoms in tubers of Russet Burbank event E12 and a decrease in symptoms in Ranger Russet event F10 compared with their respective controls. In similar tests with multiple late blight strains inoculated onto tubers, we found event G11 to be less susceptible, H50 more susceptible, and H37 variable compared with controls. After seeing these mixed results in a laboratory setting, we conducted additional field testing in 2011, using only the Ranger Russet, Russet Burbank and Atlantic varieties.
Using tubers from the 2011 field season, event E12 was found to be less susceptible to late blight than controls. In addition, Atlantic event J3 was found to be more susceptible than Atlantic controls; however, J3 was similar to Ranger Russet and Russet Burbank controls. Ranger Russet events F10 and F37 did not differ from their controls.

Late Blight Foliage Testing. In a replicated field trial in 2011, potato plants were deliberately infected with the causal agent of *P. infestans*. Of the events in that trial (E12, E24, F10, J3, J55, and J78), the only significant difference was that event F10 was more resistant than control. This replicated field trial provides evidence that Innate™ events were no more susceptible to late blight than the untransformed controls.

Soft Rot Testing with Tubers. A test with tubers in 2009 showed a similar response in controls and all events tested. Further testing in 2011 showed that Atlantic events J3 and J55 were less susceptible than untransformed controls. Considering both studies, we conclude that the events have similar susceptibility to bacterial soft rot as the controls

The studies with late blight and bacterial soft rot confirm that silencing of the target genes did not enhance susceptibility to these common diseases. As with any new variety development, we would want to avoid modifications that enhance disease susceptibility. However, if any events showed slightly higher susceptibility to disease, it would not enhance the weediness or result in the creation of plant pests.

7.5. Evidence of Trait Efficacy: Reducing Sugars

Tubers contain large amounts of phosphorylated starch, some of which is degraded during storage to produce glucose and fructose. These reducing sugars react with amino acids to form Maillard products

including acrylamide when heated at temperatures above 120^{O} C. Two enzymes involved in starch phosphorylation are water dikinase R1 and phosphorylase-L (R1 and PhL*)*. The partial or complete silencing of the associated genes in tubers can limit the accumulation of sugars, thus decreasing the potential to produce acrylamide.

The amount of reducing sugars was assessed by taking samples from either fresh or stored potatoes (**Appendix 9. Compositional Analyses**). Many of the events had lowered levels of reducing sugars either at the time of harvest or after storage for 1 month (F10, F37, E12, E24, J3, and J55, J78), however, in most cases we did not observe significant differences after 2-5 months of storage. It was not expected that J78 would have lowered levels of reducing sugars because it was one of two events, J78 and G11, that did not contain the R1/PhL promoter silencing cassette. Tubers of the events G11, H37, and H50 contain the same amount of reducing sugars as tubers of their untransformed counterparts. The inability of the silencing construct to limit glucose/fructose formation in H37 and H50 may be due to the fact that the H variety is naturally low in glucose and fructose. Thus, we concluded that silencing of the promoters associated with the *PhL/R1* genes effectively lowered reducing sugars near the time of harvest in most events but these differences were not sustained throughout storage for 2-5 months. These data indicate that partial silencing of the *R1* and *PhL* genes contributes to lower reducing sugar levels and a lowered potential to form acrylamide.

7.6. Evidence of Trait Efficacy: Black Spot Bruise

Two different methods were utilized to measure the incidence of black spot bruise, one by catechol assay and the other by physically impacting tubers using a rotating drum (**Appendix 7. Black Spot Bruise Tolerance**). Tubers of events were shown to contain either very low (F37) or undetectable activity levels (all other events, except for Atlantic events), as visualized by a catechol assay. Atlantic events were not tested because Atlantic controls did not react with the catechol assay and appeared to display some black spot bruise tolerance. The barrel bruise test indicated undetectable levels of black spot bruise in event E12, at least a 5-fold reduction in levels for events F10 and F37, and about 2-fold reduced levels in events E24, H37, and H50. The black spot bruise tolerance of event G11 showed variability depending on site.

7.7. Evidence of Trait Efficacy: Reduced Asparagine and Acrylamide

Silencing of the asparagine synthetase gene resulted in average reductions of 69 to 78% free asparagine in the potato events (**Table 8**). The lower levels of asparagine which combines with reducing sugars in the Maillard reaction to form acrylamide, results in average reductions of 58 to 72% acrylamide in fries and chips (**Table 8**). Along with the reduction in free asparagine, we have observed an increase in free glutamine, a direct result of silencing the gene that codes for the asparagine synthetase enzyme that converts glutamine to asparagine (**Figure 6**).

Table 8. Differences in Asparagine and Acrylamide Between Controls and Events

Variety	Free Asparagine (ppm)	Percent Reduction from Control	Acrylamide (ppb)[1]	Percent Reduction from Control
Ranger Control	2351	0%	371.9	0%
F10	567.2	76%	150.6	60%
F37	642.5	73%	127.3	66%
Burbank Control	2421	0%	493.8	0%
E12	618.2	74%	162.4	67%
E24	591.9	76%	158.9	68%
Atlantic Control	2268	0%	842.7	0%
J3	516.2	77%	278.7	67%
J55	502.4	78%	233.4	72%
J78	551.1	76%	251.1	70%
G Control	3406	0%	1254.2	0%
G11	794	77%	421.5	66%
H Control	2805	0%	531.2	0%
H37	877.5	69%	223.0	58%
H50	672.7	76%	174.7	67%

[1]Before testing for acrylamide, Ranger Russet and Russet Burbank controls and events were made into french fries. Atlantic, G, and H varieties were processed into potato chips. All results were from tubers analyzed near the time of harvest.

Nitrogen assimilation into amino acids occurs in all organisms and involves numerous genes and enzymes. As the primary nitrogen transport compounds within the plant, the amino acids glutamine and asparagine play crucial roles for plant growth and development (McGrath and Coruzzi 1991; Urquhart and Joy 1981). In senescing leaves, protein hydrolysis produces ammonium (Masclaux-Daubresse *et al.* 2006). Therefore, toxic ammonium must be immediately re-assimilated into organic molecules through nitrogen cycling. First, ammonium is assimilated into the glutamine amide group. Next, glutamine transfers to the position of 2-oxoglutarate, yielding two molecules of glutamate caused by the concerted reaction of the glutamine synthetase gene (*GS*). Typically, the nitrogen incorporates into other amides and amino acids, including asparagine. The glutamine-dependent enzyme asparagine synthetase (*Asn1*) catalyzes the reaction that converts aspartate to asparagine, transferring the amide nitrogen group, and converting glutamine to glutamate. The asparagine biosynthesis pathway is represented in **Figure 6**. Using Innate™ technologies, the *Asn1* gene is silenced in potato tubers, thereby allowing glutamine to retain its amide group, and to remain as glutamine in the system. This explains the reason lower asparagine levels in Innate™ potatoes create a higher level of glutamine.

Figure 6. Biosynthesis of GLN and ASN in plants

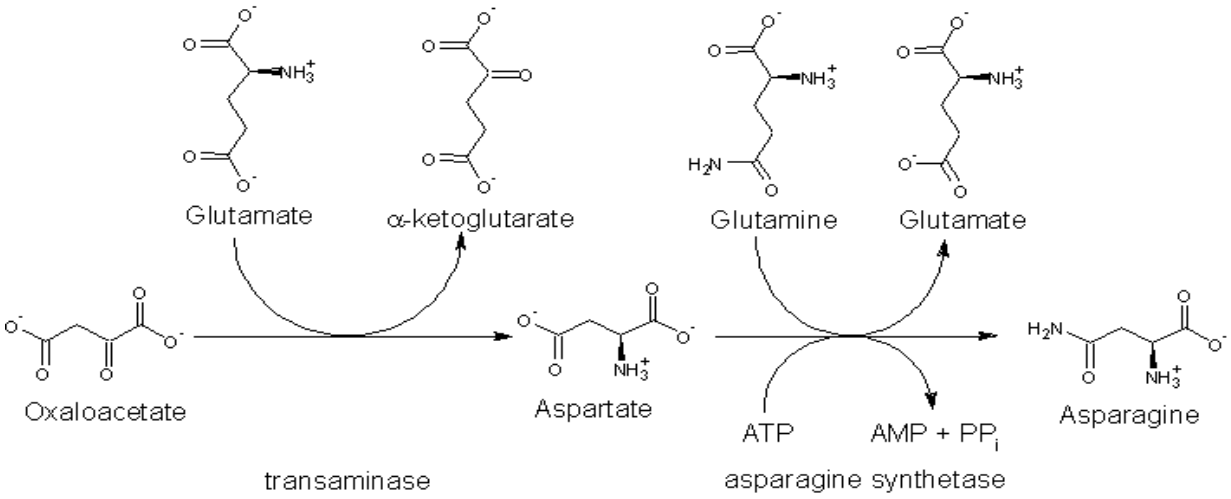


Figure 6 is from New World Encyclopedia, http://www.newworldencyclopedia.org/entry/Asparagine.

7.8. Survival in Fields

It is possible that Innate™ or conventional potatoes will persist in fields for several years after planting. In most Northern areas, potatoes are rotated with other crops which minimizes the survival in subsequent years and the chance of accidental mixing in subsequent potato plantings. However, growers who have planted Innate™ potatoes will be instructed to eliminate any potatoes persisting in the field before reverting to conventional potato production. Farmers will also be instructed not to sell waste potatoes from Innate™ plantings to small processors unless the trade restrictions with this material are clearly stated and the small processors are not engaged in potato export outside of North America.

After conducting field trials with potatoes, we routinely look for potatoes in the field the year following. We have not found any potatoes to persist in test plots from 2009 – 2011 trials after monitoring and destroying volunteers in 2010, 2011, and 2012 (to date as of this submission) (Summarized in **Appendix 10. Post-Harvest Monitoring**). These field trials consisted of small plots that are carefully monitored by researchers and represent a well-controlled environment. In large tracts of land typical of commercial potato growing operations, there might be a greater likelihood that potatoes could be overlooked. If potatoes were grown repeatedly year after year on the same ground, it would be more likely for contamination of a subsequent potato crop to occur. Extra precautions may need to be considered in those instances.

7.9. Conclusion on Agronomic Performance, Disease, and Efficacy of Traits

Transformation of potato with the DNA insert of pSIM1278 did not alter the agronomic characteristics of potatoes, and yielded the expected changes of reduced back spot bruise, lower levels of reducing sugars and reduced acrylamide potential (see **Table 9**). Reducing sugars were lowered slightly in most events, and would not be expected to be diminished in the events that did not contain the silencing construct. Significantly reduced asparagine contributed to reduced levels of acrylamide in fries and chips. The studies with late blight and bacterial soft rot confirm that silencing of the target genes did not enhance

susceptibility to these common diseases. However, if any events showed slightly higher susceptibility to disease, it would not enhance the weediness or result in the creation of plant pests.

Table 9. Traits Incorporated into Innate™ Potatoes

Event	Reduced Acrylamide Potential	Black Spot Bruise Tolerance	Reduced Accumulation of Glucose and Fructose
F10	Yes	Yes	Yes
F37	Yes	Yes	Yes
E12	Yes	Yes	Yes
E24	Yes	Yes	Yes
J3	Yes	PPO assay indicated some resistance in the Original Variety	Yes
J55	Yes	PPO assay indicated some resistance in the Original Variety	Yes
J78	Yes	PPO assay indicated some resistance in the Original Variety	Yes
G11	Yes	Yes (Variable depending on site)	No
H37	Yes	Yes	No
H50	Yes	Yes	No

Although some significant agronomic differences were observed between events and controls, most fell within the combined control range of the conventional varieties. For example, some differences in yield and tuber size were observed in events, and these attributes will be investigated further in commercial trials. Thus, we concluded that no biologically meaningful differences that would contribute to increased weediness or pest potential were observed for any of the agronomic characteristics, yield and grading characteristics, or ecological interactions (namely plant-insect interactions, plant-disease interactions, and plant interactions with abiotic stressors) of the events compared to their untransformed controls. It can be concluded that events are not different from their untransformed controls in terms of agronomy, tuber yield, and tuber composition. Therefore, the transformation of potato with pSIM1278 does not introduce characteristics that will encourage or enhance weediness or pest potential.

8. Compositional assessment

The compositional assessment studies, evaluating proximates, vitamins, amino acids, minerals, sugars, and glycoalkaloids, were conducted on Innate™ potatoes to 1) show equivalence to the untransformed controls, 2) compare the Innate™ potatoes to literature ranges, where applicable; and 3) show that there were no changes in potential toxins. Ultimately, results of the composition studies showed food safety equivalence between the events and the untransformed controls.

8.1. Proximates, Vitamins, Minerals, and Glycoalkaloids

These analyses were conducted to confirm that composition of events remained within the normal levels for potato and would have equivalent food quality, feed quality, and safety when compared to untransformed potatoes (**Appendix 9. Compositional Analyses**). We also tested for the effectiveness of gene silencing and expected to find lower amounts of asparagine and, in most cases, lower amounts of glucose and fructose compared to untransformed controls. The compositional assessments determined the amounts of 1) moisture, protein, total fat, ash, crude fiber, carbohydrate, and calories; 2) vitamins

B6, B3, and C; 3) minerals copper, magnesium, and potassium; 4) glycoalkaloids; 5) free amino acids; 6) total amino acids; and 7) reducing sugars for tubers collected from events grown in potato-growing areas of the United States. For all the nutrients listed above, the goal was to demonstrate that events contained equivalent amounts compared with the untransformed controls. Along with agronomic data, the compositional analyses helped determine whether the Innate™ potatoes exhibited compositional changes that might impact plant pest risk or food and feed safety.

Statistical analyses of nutrient composition were conducted to test for differences in the analyte mean values between each of the events and their untransformed controls. A complete description of statistical methods can be found in **Appendix 9. Compositional Analyses**. Either the P-value or adjusted P-values are provided, whereby differences $\leq$ 0.05 were considered statistically significant. Further context for data interpretation was provided through the use of tolerance intervals and published literature.

The following varieties, all grown in the various field trial sites, were used to calculate tolerance intervals: Snowden, Chieftain, Red Norland, Ida Rose, Russet Burbank, Ranger Russet, Atlantic, G, and H. The tolerance intervals were calculated to contain, with 95% confidence, 99% of the values contained in the population. This statistical 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 of all significant differences between events and controls are summarized in **Table 10** for Ranger Russet and Russet Burbank and in **Table 11** for Atlantic, G, and H varieties. Most of these differences; reduced free ASN and total ASP + ASN, increased free GLN and total GLU + GLN, reduced acrylamide, and lowered reducing sugars were expected because of the intended gene silencing. Other significant differences included decreased free VAL (J3, J55), decreased free LYS (H37), increased free ARG (F37), increased total LYS (G11), increased total PRO (G11), increased Vitamin C (F10, F37), increased niacin (F10, F37), decreased pyridoxine (J3, J55), decreased sucrose (E12, E24), and increased sucrose (H37). Both the intended changes and these observed differences fell within the tolerance intervals or combined literature values, and therefore were considered to be within normal ranges for potatoes.

It's important to note that for events E12 and E24, the free ASN levels were not statistically different from controls at the adjusted $p \leq .05$ level. However, these events showed a 74 to 76% reduction in free ASN. Such large differences were sometimes not statistically significant, which was a direct result of using the correction for False Discovery Rate (Benjamini and Hochberg 1995). These reductions were of practical significance and are considered similarly to the other events regarding this trait.

Note that free amino acids would not normally be part of a compositional assessment, however, they were included because the quantity of free asparagine is important to show the efficacy of silencing the *Asn1* gene for the purpose of reducing acrylamide. Many of the free amino acids are found at such low levels in potatoes that values barely exceed the detection limit. For the purpose of assessing the nutritional equivalence of the events, we recommend that the focus should be on comparing total amino acids to controls.

The most important changes between Innate™ tubers and their untransformed controls relates to reduction of the amino acid asparagine (ASN) and reducing sugars. Reduced amounts of free ASN and

reducing sugars in the Innate™ potatoes resulted in lowered acrylamide after heat processing compared to the conventional potatoes (see **Tables 10** and **11** as well as **Appendix 9. Compositional Analyses**).

Table 10. Statistical Differences in Composition Data: Events F10, F37, E12 and E24

Variety	Event	Attribute	Difference Expected?	Comments
Ranger Russet	F10	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Increased Niacin (B3)	No	Within TI and CLR
		Increased Vitamin C	No	Within TI and CLR
Ranger Russet	F37	Decreased Free ASN	Yes	
		Increased free ASP	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Increased Vitamin C	No	Within TI and CLR
		Increased Niacin (B3)	No	Within TI and CLR
		Increased free ARG	No	Within TI and CLR
Russet Burbank	E12	Decreased Free ASN	Yes	74% reduced, not significant
		Decreased Total ASN+ASP	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Sucrose	No	Within TI and CLR
Russet Burbank	E24	Decreased Free ASN	Yes	76% reduced, not significant
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Sucrose	No	Within TI and CLR

TI = tolerance interval
CLR = combined literature range

Table 11. Statistical Differences in Composition Data: Events J3, J55, J78, G11, H37 and H50

Variety	Event	Attribute	Difference Expected?	Comments
Atlantic	J3	Decreased Free ASN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Pyridoxine (B_6)	No	Within TI and CLR
		Decreased Free VAL	No	Within TI and CLR
Atlantic	J55	Decreased Free ASN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Pyridoxine (B_6)	No	Within TI and CLR
		Decreased Free VAL	No	Within TI and CLR
Atlantic	J78	Decreased Free ASN	Yes	Adjusted P = .0548
		Decreased Reducing Sugars	No	Glucose and Fructose
		Reduced Acrylamide	Yes	
Variety G	G11	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Reduced Acrylamide	Yes	
		Increased Total LYS	No	Within TI and CLR
		Increased Total PRO	No	Within TI and CLR
Variety H	H37	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Increased Sucrose	No	Within TI and CLR
		Reduced Acrylamide	Yes	
		Decreased Free LYS	No	Within TI and CLR
Variety H	H50	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Reduced Acrylamide	Yes	

TI = tolerance interval
CLR = combined literature range

For the compositional assessment, all events were compared with their respective controls mostly at the time of harvest. In addition, the potatoes were tested for reducing sugars and acrylamide, following storage (**Appendix 9. Compositional Analyses, <u>Storage Studies: Reducing Sugars and Acrylamide</u>**). Many of the events had lowered levels of reducing sugars either at the time of harvest or after storage for 1 month (F10, F37, E12, E24, J3, and J55, J78), however, in most cases we did not observe significant differences after 2 – 5 months of storage. Thus, we concluded that silencing of the promoters

associated with the *PhL/R1* genes effectively lowered reducing sugars near the time of harvest but these differences were not sustained throughout storage for 2-5 months.

During storage of up to 7 months, acrylamide levels in most events were consistently lower ($p < .0001$) than controls, although there were exceptions with the G and H varieties. In event G11, we observed a mean of 2,050 ppb compared with 1,769 ppb for the control after 1 month of storage. This was unusual and throughout the rest of the study, acrylamide levels in event G11 were always much lower than controls (46 to 58% reduction at months 5 and 7). For variety H, acrylamide levels were always lower in events H37 or H50, but not statistically significant in months 5 and 7. Overall, the modifications from insertion of pSIM1278 resulted in dramatic reductions in acrylamide that persisted throughout the typical storage periods.

This research also confirmed that the events are as safe as controls and did not contain increased levels of natural glycoalkaloids, which are toxins commonly found in Solanaceous crops, including potato. The principal glycoalkaloids in potatoes are α-solanine and α-chaconine. For food safety purposes, an upper limit for glycoalkaloid content of 20mg per 100g of potato is generally accepted.

The Innate™ potato events F10, F37, E12, E24, J3, J55, J78, H37, H50 and their corresponding untransformed controls had mean glycoalkaloid ranges from 5.7 to 11.3 mg/ 100g (**Table 12**); all below the 20mg/ 100g safety limit described by Sinden (1987). For one event, E24, the range of values extended to 33.81, beyond the desired limit of 20 mg/ 100 g. Although a cause was not identified, this could be from mis-handling and excessive exposure to light (Percival 1999). Since the mean value was close to the control, we would not expect that glycoalkaloid production was related to the modification.

The Innate™ potato event G11 had mean glycoalkaloids of 20.3 mg/100g and untransformed controls contained 19.8 mg/100g (**Table 12**). The higher mean levels of glycoalkaloids in the G event and untransformed controls is attributed to additional handling at the storage sites and thus, increased exposure to light and temperature flux (Percival 1999). Based on the lack of differences between the events and controls, there would not be a safety issue as a result of the introduction of the reduced acrylamide potential and low black spot bruise traits in Innate™ potatoes. All glycoalkaloid levels were within the normal ranges for potato, when compared with the tolerance intervals or combined literature ranges.

Table 12. Glycoalkaloids (mg/100 g)[1] in Ranger Russet, Russet Burbank, Atlantic, G, and H Varieties

Variety	Total Glycoalkaloids (mg/100 g)				
	Mean	Range	P-Value	TI	CLR[2]
Ranger Control	6.704	3.06-10.23		0.000-33.05	3.20-210.4
F10	6.979	2.84-9.51	0.8285		
F37	6.805	4.28-9.42	0.9812		
Burbank Control	7.404	2.74-18.59		0.000-33.05	3.20-210.4
E12	5.678	1.69-10.53	0.1707		
E24	6.845	1.45-33.81	0.8174		
Atlantic Control	6.274	2.09-11.40		0.000-33.05	3.20-210.4
J3	5.723	1.62-11.67	0.2393		
J55	5.864	1.83-11.12	0.4833		
J78	6.084	1.65-14.27	0.7198		
G Control	19.82	6.17-44.40		2.830-94.46	2.20-154.20
G11	20.32	5.81-55.3	0.9153		
H Control	10.65	5.96-21.07		1.470- 303.5	2.20-154.2
H37	10.76	5.41-17.25	0.8951		
H50	11.30	6.23-22.81	0.4379		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. P-values indicating significant differences with controls would be underlined.
[2]Literature ranges from Kozukue *et al*. (2008).

We would not expect to find novel amino acid sequences or proteins in the events because the expression cassettes consist only of non-coding DNA. There are two expression cassettes in the transferred DNA insert. One comprises an inverted repeat of fragments of asparagine synthetase-1 gene and the polyphenol oxidase-5 gene between the Agp and Gbss promoters. The other one is comprised of fragments of the promoter of the starch associated gene (R1) and the phosphorylase-L gene linked to the same Agp and Gbss promoters as the first cassette. The pool of mRNA produced by these two cassettes from either a fragment of promoters or a part of genes is unlikely to be further processed to form any amino acids or proteins. Instead, all mRNA produced by the DNA insert is processed using RNAi machinery in plant cells to silence the targeted genes: destroy mRNA of the targeted genes (Asn1, PPO) and prevent translation to form active gene products such as a protein. Therefore no changes to amino acid sequences or proteins are found with Innate™ potatoes.

8.2. Allergenicity of Innate™ Potatoes

The potato events described in this petition have the same composition compared to untransformed controls. Therefore, issues with allergies would not be expected above what individuals with potato allergies would normally have. Some allergies have been detected in children as a result of patatin contained in potatoes.

Patatin is a storage glycoprotein that displays lipase activity and makes up about 40% of the soluble protein in tubers (Mignery *et al.* 1988). Allergic reactions to processed potatoes are considered to be

very uncommon and have been reported for children only (De Swert *et al.* 2002; De Swert *et al.* 2007), with patatin (Sol t 1) identified as the major allergen involved in this reaction (Astwood *et al.* 2000). Because potato protein naturally contains a relatively large proportion of patatin, those who are allergic to potatoes would avoid them and changes to patatin levels would not alter that behavior.

8.3. Conclusions of Compositional Assessment

The intentional changes in ASN and reducing sugars accomplished through transformation of various potato varieties with the DNA insert of pSIM1278 resulted in lower acrylamide levels in french fries and potato chips. These modifications did not alter the quality of potato as food because (1) altered ASN and GLN levels are still within the normal range for potato, and (2) any impact on nutritional quality would be minimal from the observed differences in the levels of ASN, ASP, GLU and GLN because as non-essential amino acids they can be synthesized by the body. Unintentional changes in the amounts of specific amino acids, vitamin C, pyridoxine and vitamin B3 were minor and inconsistent among varieties, while resulting in levels that fell within normal ranges for potato. While one of the varieties (G) had higher levels of glycoalkaloids than expected, the events were no different from untransformed controls and were within the literature ranges reported for potato varieties. Therefore, it can be concluded that tubers from the events are as safe for consumption in food and feed as the untransformed controls.

9. Environmental Assessment of Introduction

After a thorough battery of studies to investigate the weediness potential and food and feed safety of Innate™ potatoes, the evidence submitted demonstrates that the Innate™ technologies used to introduce native traits is unlikely to harm humans, animals, or the environment.

The results of the composition analyses as described in **Appendix 9. Compositional Analyses** show overwhelming evidence of a lack of differences between Innate™ potatoes and the untransformed controls when tested for proximates, minerals, and glycoalkaloids.

The agronomic trials confirmed that the Innate™ potatoes had similar phenotypes compared with the untransformed controls when grown at multiple locations representing the major areas for potato production in the US. Observations throughout the growing season allowed for a thorough assessment of growth, ecological interactions including potential altered susceptibility to insects and diseases, and measurements of tuber yield and quality. The field assessments aided in selection of the most viable plants that contained the intentionally incorporated new traits and maintained all benefits of the conventional parent varieties (from among a larger number of events). Additionally, the field studies were used to ensure that the transformations had not resulted in the introduction of unintended effects associated with weediness or increased pest potential.

After completing the agronomic trials, potato tubers were tested for evidence of the efficacy of the traits, including an assessment for black spot bruise and reducing sugars. In addition, after processing into french fries or potato chips, the potatoes were tested for acrylamide.

Proper assessment of a biotech crop includes testing that identifies any potential negative impact on the environment. The concept of familiarity has been widely used to describe the interactions that a transformed plant may have in nature by considering the biology of the crop, the introduced trait, the receiving environment, and the interactions with all of these factors (Hokanson *et al.* 1999). This concept allows regulatory decision-makers to draw upon past experience with introduction of plants

into the environment and compare genetically engineered plants to their non-engineered counterparts. In the case of Innate™ potatoes, all of these factors were considered with respect to familiarity. Beyond the environmental factors it is also important to consider the utility of the trait. In this case, the trait reduces the level of acrylamide after frying, which can have a positive effect on human health while maintaining the typical characteristics of a potato *e.g.*, not changing farming practices or preparation of potatoes for human consumption.

9.1. Potential for Gene Transfer and Outcrossing

As previously discussed, the risks associated with accidental gene transfer in potatoes through outcrossing would be minimal for several reasons: 1) potatoes are almost always clonally propagated using "seed potatoes"; 2) a high percentage of fertile potatoes will be self-pollinated and are not frequented by honeybees due to a lack of nectar; 3) pollen transfer tends to be limited to about 20 meters which could be controlled easily in commercial fields; and 4) it is unlikely that true Innate™ seeds would grow into mature potatoes since potato seeds are not saved and propagated in the typical farming operation.

In the unlikely event that outcrossing was to occur between Innate™ and untransformed plants in the field, the impact would be negligible to future plantings since tubers rather than seed are removed from the field. If seedlings did arise from an outcrossing event, establishment would be nearly impossible since potatoes are grown in rotation and are poor competitors compared to other crop and weed species (Love 1994; CFIA 1996). If seedlings were to establish after harvest, they would be easily identified and eliminated as part of the standard agricultural practices in potato production. In either case, seedlings could be identified and eliminated to prevent them from entering the commercial stream.

In the unlikely case of growing TPS seed near the commercial Innate™ fields, a separation of 20 meters would be required to avoid outcrossing with potato events (Conner and Dale 1996).

9.2. Inter-Species Pollination and Hybridization Potential

The tetraploid species *Solanum fendleri* and diploid *Solanum jamesii* are the only wild species that grow within the borders of the United States (Bamberg *et al.* 2003; Spooner *et al.* 2004*).* These species mostly occur outside of the major potato growing regions in Texas, New Mexico, Utah, Colorado, and Arizona (see **Figure 2**). These species reside in dry forests, scrub desert, and sandy areas at altitudes of 5,000 to 10,000 feet, well isolated from most commercial production areas (Bamberg and del Rio 2011a). Therefore, pollen flow from commercial events to wild species is extremely unlikely simply because of the geographic location.

All Texas records in the IPD database are from the far western part of the state in El Paso, Culbertson and Jeff Davis Counties, where potatoes are not grown commercially. The two *S. fendleri* and one of the *S. jamesii* records are from the Davis Mountains, and one of the three *S. jamesii* entries is from Guadalupe National Park (IPD 2011; National Atlas 2004b; Hall *et al*. 2000).

The Colorado records in the IPD database are from mountainous locations very near the New Mexico border. Spooner *et al.* (2004) report that records of *S. jamesii* from natural habitats in Colorado are limited to the extreme southwestern and southeastern parts of the state. The San Luis Valley, a major

potato production area, lies between the mountainous regions where the wild potatoes were found (Bamberg *et al.* 2003; National Atlas 2004a; Spooner *et al.* 2004).

While there appears to be minimal, if any, overlap geographically between cultivated and wild potatoes in the U.S., there is a possibility that a few wild potato plants may be growing near potato fields (Love 1994). Spooner *et al.* (2004) describe *S. jamesii* habitat in the U.S. as among boulders on hillsides, sandy alluvial stream bottoms, in gravel along trails or roadways, rich organic soil of alluvial valleys, sandy fallow fields, grasslands, juniper-pinyon scrub deserts, oak thicket, coniferous and deciduous forests at elevations between 4,500 to 9,400 feet. They describe *S. fendleri* habitat similarly, and at elevations between 4,700 to 11,200 feet.

Based on conversations with Bamberg, Love (1994) reported that no one has ever reported finding hybrids between native and cultivated potatoes in the U.S., although gene transfer has been accomplished using special laboratory techniques (Love 1994). Love concluded that, based on the barriers that exist, including geographic isolation (as described above), endosperm imbalances, and multiple ploidy levels, natural hybridization is highly unlikely, and introgression (cross hybridization over multiple generations) impossible or at least highly improbable (Love 1994). More recently, the US EPA has concluded that, based on its review of the scientific literature, successful gene introgression between native and cultivated potatoes in the U.S. is virtually excluded due to constraints of geographical isolation and other barriers to natural hybridization. These barriers include incompatible (unequal) endosperm balance numbers that lead to endosperm failure and embryo abortion, multiple ploidy levels and incompatible mechanisms that do not express reciprocal genes to allow fertilization to proceed. No natural hybrids have been observed between these species and cultivated potatoes in the U.S. (US EPA 2011).

9.3. Weediness Potential

As previously described, weediness is a term used to describe the ability of a plant to become a weed (survive and thrive) outside of cultivation. The Innate™ modifications described in this petition are unlikely to increase the weediness of potato because the incorporated traits (reduced free-asparagine, black spot bruise tolerance, and reduced sugars) are not associated with weediness and will not help the plants thrive outside of cultivation. Multiple field trials of 10 events did not provide any evidence for altered growth characteristics such as accelerated tuber sprouting, increased plant vigor, increased tuber set, and delayed senescence or other key agronomic characteristics associated with survival outside of cultivation. Additionally, all fields were monitored for 2 years after study completion for volunteer activity as required by USDA compliance. Through 3 years of field studies and up-to-date volunteer monitoring of every site, no differences have been observed that would lead us to believe that the events have properties that would increase the survivability compared to conventional potatoes (see **Appendix 10. Post-Harvest Monitoring)**. Due to modern agricultural practices it is highly unlikely that potatoes would persist in a field from one crop cycle to the next, particularly since potatoes are typically grown as a rotational crop.

9.4. Endangered Species Considerations and Impact on Biodiversity

Endangered Species

Based on an assessment of threatened and endangered species in potato cropping systems, and considering the safety and compositional data of Innate™ potatoes, it is highly unlikely that there will be any effect on endangered species, the environment, or non-target organisms. Since these potatoes

introduce nothing new to the environment, *e.g.*, no new proteins or genes, they would have the same effect on endangered species as any other potatoes. Innate™ varieties are likely to be planted in areas that are already growing potatoes, and would not result in a significant expansion of acres.

Natural Biodiversity

The modifications described in this petition were intended to enhance the quality, not agronomic characteristics, of potato by reducing the expression of four native genes. Thus, we reduced the plant's ability to develop black spot bruise, store asparagine, and convert starch to sugars. Because no new pesticides are expressed, and no natural defense mechanisms have been activated, the modified potatoes have no new properties to enhance their weediness or pest resistance. The incidence of disease symptoms identified during growth and storage were similar for Innate™ and untransformed control potatoes. The Innate™ plants show no tendency to be more invasive. Invasiveness is not a trait associated with any potatoes and therefore not associated with silencing the genes affecting potato quality. These changes in potato quality have no more impact on biodiversity and endangered species than any other commercial potato varieties.

9.5. Effects on Current Agronomic Practices in Potato

Field trials with multiple potato events over three different crop years have indicated that no differences will be needed in the agronomic practices used to grow the Innate™ potatoes. The incorporated traits are not expected to affect agronomic practices. All events considered for this submission were selected to exhibit similar phenotypic characteristics to the control varieties when grown using standard industry practice. There were no differences in disease susceptibility that would require additional treatments nor did the potatoes have different nutrient requirements that would alter fertilizer programs. Planting, cultivation, management and harvesting processes were not affected by the incorporated traits. (see **Table 3** for a typical agronomic input scenario in the Northwestern United States).

9.6. Unintended or Non-target Effects

The lack of insecticidal traits in Innate™ potatoes would mean that all insects would be considered non-target. There are a large number of insects that feed on potato leaves and other insects that feed on pests. Many of these insects, including the Colorado potato beetle, potato aphid, European corn borer, potato leafhopper, and potato psyllid are considered pests. The events have not been modified to display any new pesticidal activities and do not contain new pesticide-expressing genes. No differences were observed in the field trials for insects or other animals interacting within the potato ecosystem proving a lack of pest potential to other organisms. The only modifications concerned the intended effects of reduced transcript levels for four quality-associated genes, mainly in tubers and stolons. During the three seasons of field releases, the observations and measurements showed no differences in ecological interactions (namely plant-insect interactions, plant-disease interactions, and plant interactions with abiotic stressors) between the events and the control varieties (**Appendix 6. Field Performance and Tuber Evaluations**), supporting an absence of unintended effects.

9.7. Summary of the Environmental Assessment of Introduction

The modifications described in this petition were intended to enhance the quality, not agronomic characteristics, of potato by inactivating four native genes. Planting, cultivation, management and harvesting techniques were not affected by the incorporated traits. The Innate™ varieties are likely to be planted in areas that are already growing potatoes, and would not result in a significant expansion of

acres. The modification described in this petition is highly unlikely to increase the weediness or invasiveness of potato because the incorporated traits (reduced free-asparagine, black spot bruise tolerance, and reduced sugars) do not play a role in survival, dispersal, or ecological competitiveness. Field trials over multiple years with 10 events did not provide any evidence for altered growth characteristics such as accelerated tuber sprouting, increased plant vigor, increased tuber set, or delayed senescence. No new pesticides are expressed, and no natural defense mechanisms activated, and thus modified potatoes are unlikely to display enhanced weediness or pesticidal properties. Therefore, the potential to impact insects and other non-target organisms, weed or disease susceptibility, endangered species or biodiversity is negligible for Innate™ potatoes.

The data presented in this petition demonstrate that introduction of Innate™ potatoes will have a similar environmental impact when compared to untransformed potatoes. Use of Innate™ technologies and the resultant transformed plants, and their tuber products, satisfy the statutory requirements for nonregulated status in that our transformants have no pesticidal traits and are not plant pests. To conclude, we feel that the plants containing these traits, are not plant pests and have resulted from the addition of genetic material from a donor plant where the integrated genetic material contains only non-coding regulatory regions, that are well characterized, and are as safe as untransformed potatoes. The evidence presented in this submission demonstrates that the deregulation and cultivation of Innate™ potatoes is highly unlikely to cause any adverse environmental or biological impacts as a result of their cultivation.

10. Crop Introduction and the Potato Industry

10.1. Industry Need for Potatoes with Reduced Acrylamide Potential

Based on a recent notice (Federal Register 2009), FDA is considering issuing guidance for industry on the reduction of acrylamide levels in food products. The introduction of Innate™ potatoes with low acrylamide potential would provide potatoes that are largely indistinguishable from existing varieties. The reduction in asparagine and sugars using Innate™ technologies and the resulting reduction in acrylamide upon heating will address industry needs with respect to the proposed FDA guidance.

In addition, recent litigation in the state of California resulted in legal settlements with restaurant chains, and the retail french fry and potato chip manufacturers regarding acrylamide. The settlements required manufacturers of retail fries and potato chips to reduce the level of acrylamide in their products. Potato processors affected by these rulings in California could be motivated to adopt the low acrylamide, Innate™ potato events.

The low acrylamide potatoes will provide a safer option for all sectors of the potato processing market and thus should increase the demand for these Innate™ potatoes. The benefits resulting from the modifications could result in increased market share, particularly for Ranger Russet and Atlantic. The reduction of sugars and black spot bruise in Ranger Russet may increase the popularity of that variety.

10.2. Potato uses and exports

Potatoes are grown commercially in 36 of the 50 states in the US. Total US production in 2010 was 40.4 billion lbs, with total value of $3.72 billion, planted on 1.03 million acres (NPC 2012). The commercial uses include 37% as frozen, 27% sold fresh, 14% chips, 8% dehydrated, 5% seed potatoes, and 1% canned (NPC 2012). Annual per capita consumption in the US was expected to be approximately 112 lbs per person in 2012 (NPC 2012).

The states with the largest volume and sales value in 2009 were Idaho, Washington, Wisconsin, Colorado, Oregon, Minnesota, North Dakota, Michigan, Maine, and California (USDA-NASS 2010b). These data represent the combined markets including all processing and fresh varieties.

During the 2009/10 marketing year (September-August), US exports of all potatoes and potato products (including starch) totaled $1.19 billion—essentially unchanged from a year earlier (USDA ERS 2010). Japan remained the top foreign market for U.S. potatoes with 28% of the total export value; primarily frozen french fries, other frozen potato products, potato flakes and granules. Japan was followed by Canada (24 % of export value), Mexico (11 %), South Korea (5 %), and China (4 %).

Potato exports from the U.S. in 2010 totaled 5.7 billion lbs (farm weight of potatoes) and 812 million lbs of fresh potatoes. Most of the processed potato exports were frozen, representing 51.4% of total farm weight production (USDA ERS 2010).

10.3. Submissions to Other Regulatory Agencies

Approval for these events will be sought in Canada, Japan, Mexico, and the US. These approvals should ensure continued trade with key export markets.

10.4. Impact on the Organic Market

Organic potatoes in 2008 represented less than 1.0% (0.7%) of total potato production with 8,273 acres of certified organic potatoes (USDA ERS 2011). Of the 8,273 organic acres, California, Washington, and Oregon were the top three organic potato producers with 3,697 acres, 1,600 acres, and 1,101 acres, respectively. Potato varietal selection and breeding are similar between organic and conventional potato production as commercial potatoes are predominantly planted from tuber seed. Risks to organic growers would be most likely to occur with accidental mixing of planting material or of potatoes in farming, transportation, or processing channels. These risks are the same as those that organic growers already experience when keeping their organically grown potatoes separate from conventionally grown potatoes. Because potatoes are clonally propagated, the risk of contaminating seed supplies through cross-pollination is negligible. Organic farmers routinely plant organic tuber seed material and any incidence of cross-pollination in production fields will not affect the harvested potatoes. Producers of organic true potato seed will be able to protect their seed from Innate™ material by ensuring that the seed production fields are sufficiently isolated from Innate™ potato fields. Potential outcrossing can be prevented by separating fields planted for organic TPS production by at least 20 meters from fields planted with Innate™ potatoes (Conner and Dale 1996).

10.5. History of Biotech Potatoes

A review of the history of the introduction of genetically modified potatoes in 1995 in the U.S. (Thornton 2008) indicates the importance of a careful assessment of the performance of Innate™ events, synchronizing approvals in North American and trading countries, and weathering public acceptance issues that may result from activism against food industry members that adopt the new technology.

This history points to the importance of consumer acceptance when introducing any Innate™ crops and products to the market. Since the time when biotech potatoes were on the market briefly, the adoption of biotechnology in the food supply has increased consistently. Herbicide tolerant soybeans have increased from 54% to 93% of the U.S. soybean acreage (USDA-NASS 2010a) since 2000. Genetically modified (GM) corn production has increased from 25% of U.S. corn acreage in 2000 to 86 % of corn acreage in 2010 (USDA-NASS 2010a). These two crops provide significant quantities of GM-derived ingredients in widely marketed processed food and feed products, such as protein, oils, starches, and sweeteners. Many of the conventional processed potato products made from the events presented in this petition already contain one or more of these GM ingredients. Importantly, the low acrylamide potato events contain a new trait that has value to the consumer. The growing interest in improving food safety by reducing acrylamide in processed potatoes is expected to provide an incentive for enhanced consumer acceptance of these new events and the products derived from them.

10.6. Stewardship of Innate™ Potatoes

Stewardship of Potato Events

Throughout development of the Innate™ potatoes, the Biotechnology Quality Management System (USDA-BRS Compliance assistance program) has served as a stewardship program to conduct field trials, monitor movement, and complete testing covering the duration of the regulated status of Innate™ potatoes. As a part of BQMS, qualified and experienced potato growers were identified, and agreements were established to ensure compliance and conformance requirements for growing Innate™ potatoes. Processes were developed that included Standard Operating Procedures (SOPs) and forms for all critical control points to guide internal and external collaborators to conduct activities using Innate™ potatoes.

The following management procedures were included for effective implementation of BQMS:

1. Document and record control
2. Resource management
3. Training external and internal associates
4. Planning and process realization
5. Conducting internal audits
6. Implement corrective / preventive actions
7. Continuous improvement of the system
8. Resolution of compliance issues

Targeted training programs were used to educate and bring awareness on handling Innate™ potatoes in storage, transport, planting, harvest, post-harvest, processing, and final disposition to ensure no Innate™ potato material entered into the food or feed channels prior to de-regulation. Both internal and external audits were used to verify that planned activities occurred and personnel were adhering to the protocols recommended by BQMS. This stewardship program includes corrective and preventive actions

to avoid compliance and conformance incidents. While BQMS served as a quality management system during product development, we anticipate a full extension of such methods as outlined in the 'Excellence Through Stewardship®' (BIO 2007) program.

Identity Preservation
To help prevent the trade disruptions experienced with other potato events, international approvals will be pursued from key trading partner countries before the Innate™ varieties are launched commercially. The initial Innate™ introduction will build up slowly as seed becomes available and will be controlled within existing processing channels to ensure that potatoes enter only the intended markets. This will provide an extended period of time to assess consumer acceptance and to address grower and industry awareness. A limited introduction in a vertically integrated supply chain will be well controlled by grower and processor agreements. In this situation, conventional products will be considered "identity preserved" with respect to the well-controlled stewardship of the Innate™ crop and its products. As Innate™ potato adoption increases, programs for identity preservation will be implemented as needed. It's expected that development and implementation of identity preservation systems will add some cost to the supply chain. The total costs will depend upon the type and extent of market penetration.

Synchronized Regulatory Approvals
We intend to follow the recommended stewardship policy statement released by the Biotechnology Industry Organization in May 2007.

"To help ensure the continued adoption of agricultural biotechnology globally and to continue to have products of agricultural biotechnology bring value to the marketplace, BIO's Food and Agriculture Section supports actions that facilitate the flow of goods in commerce and minimize trade disruptions. BIO's Food and Agriculture Section believes that henceforth individual member companies should, prior to commercialization meet applicable regulatory requirements in key countries identified in a market and trade assessment that have functioning regulatory systems and are likely to import the new biotechnology-derived plant products."

The voluntary guideline was adopted because, according to BIO: "asynchronous authorizations combined with importing countries maintaining "zero tolerance" for recombinant-DNA products not yet authorized results in the potential for major trade disruptions. The potential occurrences of trade disruptions will only increase given the substantial amount of research that will bring many new products and combinations of products to market."

The submission for nonregulated status in the US will be followed by applications submitted in Canada, Mexico, and Japan, with the intention of completing approvals before crop introduction.

11. Conclusions: Determination of Nonregulated Status for Innate™ Potatoes

Simplot's Innate™ technologies allow us to transform plants with potato DNA and only introducing non-coding DNA (DNA not coding for RNA that is translated into protein) into the plant's genome. We transformed five different varieties with pSIM1278, and selected ten of these events for determination of nonregulated status: F10 and F37 for Ranger Russet, E12, and E24 for Russet Burbank, J3, J55, and J78 for Atlantic, G11 for variety G, and H37 and H50 for variety H. As demonstrated in the data presented in this Petition, the transformed cultivated potato plants are not parasitic, and our data prove there is no reason to believe they are plant pests. Furthermore, the expression of the integrated genetic elements results in traits highly sought-after in the potato industry, namely: reduced black spot bruise, low

asparagine, and lower levels of reducing sugars. Ultimately, we demonstrate that the Innate™ tubers exhibited the desired traits which can lead to lower acrylamide levels in cooked potatoes and, importantly, we showed that the transformed tubers were otherwise substantially equivalent to untransformed controls.

To conclude, we have created plants utilizing biotechnology that are not plant pests and which have resulted from the addition of well characterized non-coding regions from potato or wild potato that are as safe as untransformed potatoes. We therefore seek nonregulated status of potato plants transformed using our Innate™ technologies and submit the evidence that these plants should not be classified as "Regulated articles" as defined under 7 CFR 340.

12. Statement of Grounds Unfavorable

JR Simplot is not aware of any information indicating that Innate™ potatoes may pose a greater plant pest risk than conventional potatoes. There are no adverse environmental consequences anticipated with its introduction based on the data collected to date. The benefits of introduction of commercial events are provided in this submitted petition.

13. Certification

The undersigned certifies that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioners which are unfavorable to the petition.

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Boise, ID 83706

14. Record of Field Test Reports

The J.R. Simplot Company has adhered to USDA compliance regulations by submitting the field test reports for each notification (**Table 13**). Volunteer monitoring for 2009, 2010, and 2011 field trials is discussed in **Appendix 10** (**Post Harvest Monitoring**).

Table 13. Field release notifications

USDA Reference Number	Field Trial Year	Effective Start and Expiration Date	Release Sites (by State) Covered by Notification	Field Test Report Submitted (Yes or No)
08-353-103n	2009	1/30/2009 – 1/30/2010	FL	Yes
08-353-104n	2009	4/01/2009 – 4/01/2010	MI	Yes
09-049-114n	2009	4/13/2009 – 4/13/2010	ID, WI	Yes
09-077-112n	2009	4/23/2009 – 4/23/2010	NE	Yes
09-336-103n	2010	1/25/2010 – 7/31/2011	FL	Yes
10-053-132n	2010	4/05/2010 – 4/05/2011	ID, MI, ND, NE, WA, WI	Yes
10-076-103n	2010	4/26/2010 – 4/26/2011	WI	Yes
10-326-103n	2011	1/03/2011 – 1/03/2012	FL	Yes
11-063-103n	2011	4/04/2011 – 4/03/2012	ID, IN, MI, ND, NE, WA, WI	Yes
11-094-106n	2011	5/02/2011 – 5/2/2012	WI	Yes
11-150-101n	2011	6/06/2011 – 6/62012	MI	Yes
11-083-111n	2011	4/11/2011 – 4/11/2012	ID	Yes
11-074-109n	2011	4/04/2011 – 4/04/2012	WA	Yes
12-018-110n	2012	2/01/2012 – 2/01/2013	ID	No

15. Literature Cited

Astwood J, Alibhai M, Lee T, Fuchs R, Sampson H (2000) Identification and characterization of IgE binding epitopes of patatin, a major food allergen of potato. Program and abstracts of the 56th annual meeting of the American Academy of Allergy, Asthma and Immunology, March 3-8, San Diego, Calif. Abstracts 555.

Bamberg JB, del Rio A, Huaman Z, Vega S, Martin M, Salas A, Pavek J, Kiru S, Fernandez C, Spooner D (2003) A decade of collecting and research on wild potatoes of the southwest. Am J of Potato Res, 80: 159-172.

Bamberg JB, del Rio A (2011a) Use of native potatoes for research and breeding. HortScience, 46: 1444-1445.

Bamberg JB, del Rio A (2011b) Diversity relationships in tetraploid wild potato native to the USA [abstract]. Am J of Potato Res, 88:29. http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=252056

Bartlett JG, Alves SC, Smedley M, Snape JW, Harwood WA (2008) High-throughput *Agrobacterium*-mediated barley transformation. Plant Methods, 4: 22.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B (Methodological), 57: 289–300.

BIO (2007) US Biotechnology Industry Organization Product Launch Stewardship Policy. http://www.bio.org/foodag/stewardship/20070521.asp (Accessed 22 May 2011).

CFIA (1996) Biology Document BIO1996-09: The Biology of *Solanum tuberosum* (L.) (Potatoes). Canadian Food Inspection Agency. http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/solanum-tuberosum-l-/eng/1330982063974/1330982145930 (Accessed 23 August 2012).

Chawla R, Shakya R, Rommens CM (2012) Tuber specific silencing of asparagine synthetase-1 reduces the acrylamide-forming potential of potatoes grown in the field without affecting tuber shape and yield. Plant Biotechnol J, 10: 913-924.

Cheng M, Fry JE, Pang SZ, Zhou HP, Hironaka CM, Duncan DR, Conner TW, Wan YC (1997) Genetic transformation of wheat mediated by *Agrobacterium* tumefaciens. Plant Physiol, 115: 971–980.

Chiou TJ, Aung K, Lin SI, Wu CC, Chiang SF, Su CI (2006) Regulation of phosphate homeostasis by microRNA in Arabidopsis. Plant Cell, 18: 412-421.

Conner AJ, Dale PJ (1996) Reconsideration of pollen dispersal data from field trials of transgenic potatoes. Theor Appl Genet, 92: 505-508.

Conner AJ, Mlynárová L, Stiekema WJ, Nap JP (1998) Meiotic stability of transgene expression is unaffected by flanking matrix-associated regions. Mol Breeding, 4: 47-58.

Courvalin P, Weisblum B, Davies J (1977) Aminoglycoside-modifying enzyme of an antibiotic-producing bacterium acts as a determinant of antibiotic resistance in Escherichia coli. P Natl Acad Sci USA, 74: 999-1003.

Daxinger L, Hunter B, Sheikh M, Jauvion V, Gasciolli V, Vaucheret H, Matzke M, Furner, I (2008) Unexpected silencing effects from T_DNA tags in Arabidopsis. Trends Plant Sci, 13: 4-6.

De Buck S, Jacobs A, Van Montagu M, Depicker A (1999) The DNA sequences of T-DNA junctions suggest that complex T-DNA loci are formed by a recombination process resembling T-DNA integration. Plant J, 20: 295-304.

De Buck S, Podevin N, Nolf J, Jacobs A, Depicker A (2009) The T-DNA integration pattern in Arabidopsis transformants is highly determined by the transformed target cell. Plant J, 60: 134-145.

De Neve M, De Buck S, Jacobs A, Van Montagu M, Depicker A (1997) T-DNA integration patterns in co-transformed plant cells suggest that T-DNA repeats originate from ligation of separate T-DNAs. Plant J, 11: 15–29.

De Swert LF, Cadot P, Ceuppens JL (2002) Allergy to cooked white potatoes in infants and young children: A cause of severe, chronic allergic disease. J Allergy Clin Immun, 110: 524-535.

De Swert LF, Cadot P, Ceuppens JL (2007) Diagnosis and natural course of allergy to cooked potatoes in children. Allergy, 62: 750-757.

Dwelle, RB (2003) Potato growth and development. In: Potato Production Systems (Eds. Stark JC and Love S) University of Idaho Agricultural Communications, Moscow, pp. 9-19.

Federal Register (2009) “Acrylamide in Food, Request for Comments and for Scientific Data and Information.” *Federal Register* 74 (26 August 2009): 43134-43138. Print.

Federal Register (2011) ”Pesticides; Data Requirements for Plant-incorporated Protectants (PIPs) and Certain Exemptions for PIPs; Notification to the Secretaries of Agriculture and Health and Human Services, Proposed Rule.” *Federal Register* 76 (16 March 2011): 14358-14359. Print.

Garbarino JE, Belknap WR (1994) Isolation of a ubiquitin-ribosomal protein gene (ubi3) from potato and expression of its promoter in transgenic plants. Plant Mol Biol, 24: 119-127.

Gelvin SB (2003) *Agrobacterium*-mediated plant transformation: the biology behind the "gene-jockeying" tool. Microbiol. Mol Biol R, 67: 16-37.

Gheysen G, Villarroel R, Van Montagu M (1991) Illegitimate recombination in plants: a model for T-DNA integration. Gene Dev, 5: 287-297.

Gutiérrez RA, Stokes TL, Thum K, Xu X, Obertello M, Katari MS, Tanurdzic M, Dean A, Nero DC, McClung CR, Coruzzi GM (2008) Systems approach identifies an organic nitrogen-responsive gene

network that is regulated by the master clock control gene CCA1. P Natl Acad Sci, 105: 4939-4944.

Hagio M, Sakurai I, Sato S, Kato T, Tabata S, Wada H (2002) Phosphatidyglycerol is essential for the development of thylakoid membranes in Arabidopsis thaliana. Plant Cell Physiol, 43: 1456-1464.

Hakimi SM, Krohn BM, Stark DM: Monsanto Technology LLC (2006) United States Patent: Method of imparting disease resistance to plants by reducing polyphenol oxidase activities. Patent No.: US 7,122,719 B2.

Hall KD, Holloway RL, Smith DT (2000) Texas Crop Profile: Potatoes. Texas Agricultural Extension Service, Texas A & M University, TX.

Hanneman RE (1994) The testing and release of transgenic potatoes in the North American Center of diversity. In: Biosafety of Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere (Eds. Krattiger AF and Rosemarin A). ISAAA, Ithaca and SEI, Stockholm, pp. 47-67.

Hijmans RJ, Spooner DM (2001) Geographic distribution of wild potato species. Am J of Botany, 88: 2101-2112.

Hokanson K, Heron D, Gupta S, Koehler S, Roseland C, Shantharam S, Turner J, White J, Schechtman M, McCammon S, Bech R (1999) The concept of familiarity and pest resistant plants. Proceedings of a workshop on: Ecological Effects of pest resistance genes in managed ecosystems. January 31-February 3, 1999, Bethesda, MD. USDA-APHIS Plant Protection and Quarantine, Scientific Services.

Hopkins BG, Stark J, Hafez SL, Hutchinson PJS, Patterson P, Miller J, Thornton M, and Alvarez, J (2003) Field Selection, Crop Rotations, and Soil Management. In: Potato Production Systems (Eds. Stark JC and Love S) University of Idaho Agriculutral Communications, Moscow, pp. 1-7.

IPD (2011) Inter-genebank Potato Database. Wild Potato Collection. http://research.cip.cgiar.org/IPD/ (Accessed 10 November 2011).

Kim SI, Veena, Gelvin SB (2007) Genome-wide analysis of *Agrobacterium* T-DNA integration sites in the Arabidopsis genome generated under non-selective conditions. Plant J, 51: 779-791.

Kononov ME, Bassuner B, Gelvin SB (1997) Integration of T-DNA binary vector 'backbone' sequences into the tobacco genome: evidence for multiple complex patterns of integration. Plant J, 11: 945-957.

Kozukue N, Yoon KS, Byun GI, Misoo S, Levin CE, Friedman M (2008) Distribution of glycoalkaloids in potato tubers of 59 accessions of two wild and five cultivated Solanum species. J Agric Food Chem, 56: 11920-11928.

Lazo GR, Stein PA, Ludwig RA (1991) A DNA transformation-competent Arabidopsis genomic library in *Agrobacterium*. Biotechnology, 9: 963-967.

Lechtenberg B, Schubert D, Forsbach A, Gils M, Schmidt R (2003) Neither inverted repeat T-DNA configurations nor arrangements of tandemly repeated transgenes are sufficient to trigger transgene silencing. Plant J, 34: 507-517.

Li L, Steffens JC (2002) Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. Planta, 215: 239-247.

Llorente B, Alonso GD, Bravo-Almonacid F, Rodríguez V, López MG, Carrari F, Torres HN, Flawiá MM (2011) Safety assessment of nonbrowning potatoes: opening the discussion about the relevance of substantial equivalence on next generation biotech crops. Plant Biotechnol J, 9: 136-150.

Lorberth R, Ritte G, Willmitzer L, Kossmann J (1998) Inhibition of a starch-granule-bound protein leads to modified starch and repression of cold sweetening. Nat Biotechnol, 16: 473-477.

Love S (1994) Ecological risk of growing transgenic potatoes in the United States and Canada. Am Potato J, 71: 647-658.

Lusk JL, Rozan A (2006) Consumer acceptance of ingenic foods. Biotechnol J, 1: 1433-1434.

Lusk JL, Sullivan P (2002) Consumer acceptance of genetically modified foods. Food Technology, 56: 32-37.

Masclaux-Daubresse C, Reisdorf-Cren M, Pageau K, Lelandais M, Grandjean O, Kronenberger J, Valadier MH, Feraud M, Jouglet T, Suzuki A (2006) Glutamine synthetase- glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink-source nitrogen cycle in tobacco. Plant Physiol, 140: 444-456.

Mayer AM (2006) Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry, 67: 2318-2331.

McGrath RB, Coruzzi GM (1991) A gene network controlling glutamine and asparagines biosynthesis in plants. The Plant J, 1: 275-280.

Mignery GA, Pikaard CS, Park WD (1988) Molecular characterization of the patatin multigene family of potato. Gene, 62: 27-44.

Müller AJ, Mendel RR, Schiemann J, Simoens C, Inzé D (1987) High meiotic stability of a foreign gene introduced into tobacco by *Agrobacterium*-mediated transformation. Mol Gen Genet, 207: 171-175.

Nakata PA, Anderson JM, Okita TW (1994) Structure and expression of the potato ADP-glucose pyrophosphorylase small subunit. J Bio Chem 269: 30798-30807.

National Atlas (2004a) "Colorado." *The National Atlas of the United States of America*. 2004. http://www.nationalatlas.gov (Accessed 11 November 2011).

National Atlas (2004b) "Texas." *The National Atlas of the United States of America*. 2004. http://www.nationalatlas.gov (Accessed 12 November 2011).

NPC (2012) National Potato Council, 2012 Potato Statistical Yearbook.

NTP (2012) Technical Report on the Toxicology and Carcinogenesis Studies of Acrylamide in F344/N Rats and B6C3F1 Mice. National Toxicology Program. National Institutes of Health. Public Health Service. U.S. Dept. of Health and Human Services.

Obrien J, Morrissey PA (1989) Nutritional and toxicological aspects of the Maillard browning reaction in foods. Crit Rev Food Sci, 38: 211-248.

OECD (1997) Organization for Economic Co-Operation and Development, Consensus document on the biology of *Solanum tuberosum* subsp*. tuberosum* (potato), 38 pages.

OEHHA (2011a) Acrylamide: frequently asked questions. Office of Environmental Health Hazard Assessment. http://www.oehha.ca.gov/prop65/acrylamideqaall.pdf (Accessed 24 May 2011).

OEHHA (2011b) Proposition 65: Acrylamide (2011). Office of Environmental Health Hazard Assessment. http://www.oehha.ca.gov/prop65/acrylamide.html (Accessed 24 May 2011).

Oltmanns H, Frame B, Lee LY, Johnson S, Li B, Wang K, Gelvin SB (2010) Generation of backbone-free, low transgene copy plants by launching T-DNA from the *Agrobacterium* chromosome. Plant Physiol, 152: 1158-1166.

Pallais N, Mulcahy D, Fong N, Falcon R, Schmiediche P (1988) The relationship between potato pollen and true seed: effects of high temperature and pollen size. In Sexual Reproduction in Higher Plants. Spinger-Verlag, Berlin Heidelberg.

Percival G (1999) Light-induced glycoalkaloid accumulation of potato tubers (*Solanum tuberosum L*). J Sci Food Agric, 79: 1305-1310.

Plaisted RL (1980) Potato. In: Hybridization of crop plants (Eds. Fehr, W.R. and Hadley, H.H.). American Society of Agronomy and Crop Science Society of America, publishers, Madison, Wisconsin, 483-494.

Prescott EM, Proudfoot NJ (2002) Transcriptional collision between convergent genes in budding yeast. P Natl Acad Sci USA, 99: 8796-8801.

Ramon M, Rolland F, Sheen J (2008) Sugar Sensing and Signaling. The Arabidopsis Book (TAB), ISSN: 1543-8120 http://www.aspb.org/publications/arabidopsis/pages 1-22.

Richael CM, Kalyaeva M, Chretien RC, Yan H, Adimulam S, Stivison A, Weeks JT, Rommens CM (2008) Cytokinin vectors mediate marker-free and backbone-free plant transformation. Transgenic Res, 17: 905-917.

Sallaud C, Meynard D, van Boxtel J, Gay C, Bès M, Brizard JP, Larmande P, Ortega D, Raynal M, Portefaix M, Ouwerkerk PB, Rueb S, Delseny M, Guiderdoni E (2003) Highly efficient production and characterization of T-DNA plants for rice (Oryza sativa L.) functional genomics. Theor Appl Genet, 106: 1396-1408.

Sinden SL (1987) Potato glycoalkaloids. Acta Hortic., 207: 41-48.

Skirvin RM, Norton M, McPheeters KD (1993) Somaclonal variation: has it proved useful for plant improvement? Acta Horticulturae, 336: 333-340.

Smigocki AC, Owens LD (1988) Cytokinin gene fused with a strong promoter enhances shoot organogenesis and zeatin levels in transformed plant cells. P Natl Acad Sci USA, 85: 5131-5135.

Spooner DM, van den Berg RG, Rodriguez A, Bamberg JB, Hijmans RJ, Cabrera SIL (2004) Wild potatoes (*Solanum* section *Petota*: *Solanaceae*) of North and Central America. Syst Bot Monogr, 68: 1-209.

Spooner DM, McLean K, Ramsay G, Waugh R, Bryan GJ (2005) A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. P Natl Acad Sci USA, 102: 14694-14699.

Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert MC, Riediker S (2002) Acrylamide from Maillard reaction products. Nature, 419: 449-50.

Steffens JC, Harel E, Hunt MD (1994) Polyphenol Oxidase. In: Genetic Engineering of Plant Secondary Metabolism (Ed. Ellis BE) Plenum Press, New York, pp. 275-312.

Thipyapong P, Hunt MD, Steffens JC (2004) Antisense downregulation of polyphenol oxidase results in enhanced disease susceptibility. Planta, 220: 105-117.

Thipyapong P, Stout MJ, Attajarusit J (2007) Review: Functional analysis of polyphenol oxidases by antisense/sense technology. Molecules, 12: 1569-1595.

Thornton M (2008) The rise and fall of NewLeaf™ potatoes. http://nabc.cals.cornell.edu/pubs/nabc_15/chapters/Thornton.pdf (Accessed 31 May 2011).

Tuteja JH, Zabala G, Varala K, Hudson M, Vodkin LO (2009) Endogenous, tissue-specific short interfering RNAs silence the chalcone synthase gene family in glycine max seed coats. Plant Cell, 21: 3063-3077.

Urquhart AA, Joy KW (1981) Use of phloem exudates technique in the study of amino acid transport in pea plants. Plant Physiol, 68: 750-754.

US EPA (2011) United States Environmental Protection Agency. Introduction to Biotechnology Regulation for Pesticides. http://epa.gov/oppbppd1/biopesticides/regtools/biotech-reg-prod.htm (Accessed 27 January 2012).

USDA ERS (2010) Vegetables and Melons Outlook. United States Department of Agriculture Economic Research Service. http://www.ers.usda.gov/publications/vgs (Accessed 2 April 2012).

USDA ERS (2011) Organic Production. United States Department of Agriculture Economic Research Service. http://www.ers.usda.gov/Data/Organic (Accessed 15 June 2011).

USDA-NASS (2010a) United States Department of Agriculture, National Agricultural Statistics Service. Acreage from June 2000-June 2010 (Accessed 24 May 2011).

USDA-NASS (2010b) United States Department of Agriculture, National Agricultural Statistics Service. Potatoes 2009 Summary ISSN: 1949-1514 (Accessed 24 May 2011).

Valentines MC, Vilaplana R, Torres R, Usall J, Larrigaudiere C (2005) Specific roles of enzymatic browning and lignifications in apple disease resistance. Postharvest Biotech and Tech, 36: 227-234.

Van Haaren MJJ, Sedee NJA, de Boer HA, Schilperoort RA, Hooykaas PJJ (1989) Mutational analysis of the conserved domains of a T-region border repeat of Agrobacterium tumefaciens. Plant Mol Bio, 13: 523-531.

Visser RGF, Stolte A, Jacobsen E (1991) Expression of a chimeric granule-bound starch synthase-GUS gene in transgenic potato plants. Pant Mol Bio, 691-699.

Webb RE, Wilson DR, Shumaker JR, Graves B, Henninger NR, Watts J, Frank JA, Murphy HJ (1978) Atlantic: a new potato variety with high solids, good processing quality, and resistance to pests. Am Potato J, 55: 141-145.

Windels P, DeBuck S, VanBockstaele E, DeLoose M, Depicker A (2003) T-DNA Integration in Arabidopsis Chromosomes. Presence and Origin of Filler DNA Sequences. Plant Physiol, 133: 2061-2068.

Wolters AA, Trindade LM, Jacobsen E, Visser RGF (1998) Fluorescence in situ hybridization on extended DNA fibres as a tool to analyse complex T-DNA loci in potato. Plant J, 13: 837-847.

Yan H, Chretien R, Ye J, Rommens CM (2006) New construct approaches for efficient gene silencing in plants. Plant Physiol, 141: 1508-1518.

Yang L, Ding J, Zhang C, Jia J, Weng H, Liu W, Zhang D (2005) Estimating the copy number of transgenes in transformed rice by real-time quantitative PCR. Plant Cell Rep, 23: 759-763.

Zhu W, Ouyang S, Iovene M, O'Brien K, Vuong H, Jiang J, Buell CR (2008) Analysis of 90 Mb of the potato genome reveals conservation of gene structures and order with tomato but divergence in repetitive sequence composition. BMC Genomics, 9:286.

Appendix 1
Characterization of Inserted DNA

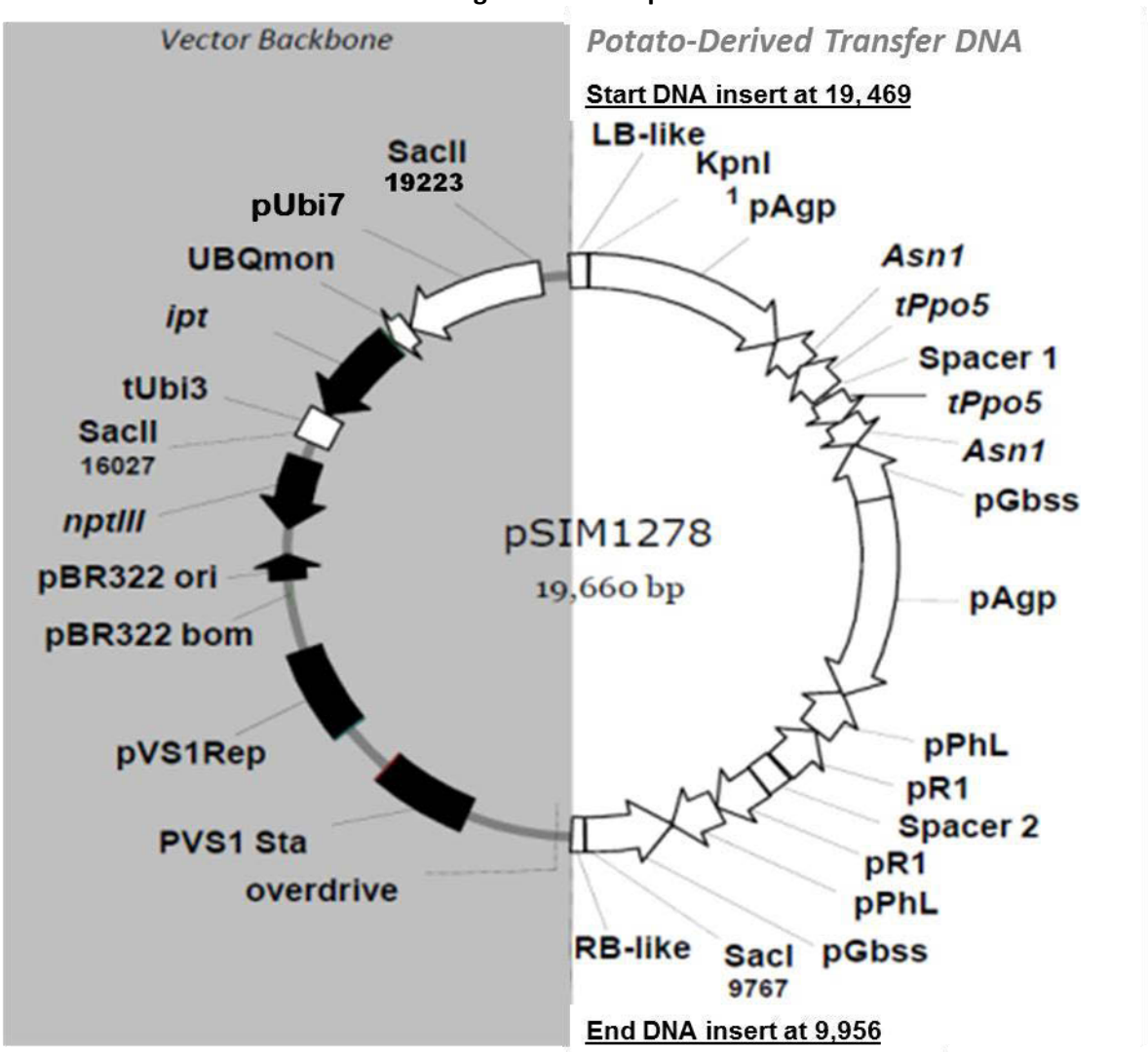
INDEX

INTRODUCTION

Events were evaluated by DNA gel blot analyses to determine the structure and copy number of the integrated DNA insert (vector map presented in **Figure 1**). The descriptions of the genetic elements are shown in **Table 1.** These studies were carried out as parts of the characterization and biosafety assessment of the events.

DNA gel blot analysis was performed using DNA extracted from greenhouse-grown plants and digested with *Eco*RV, *Hin*dIII, or *Eco*RI + *Sca*I. The digested DNA was run with standard DIG-labeled molecular weight markers, DIGII and DIGVII, through 0.7% agarose gels in 0.5X TBE buffer and blotted onto Nylon Hybond N+ filters. The DNA insert-derived probes used were named ASN (from the *Asn1/Ppo5* gene silencing cassette), AGP, AGP2 and AGP3 (all from the Agp promoter driving both silencing cassettes), GBS (from the Gbss promoter driving both silencing cassettes), and PHL (from the R1/PhL promoter silencing cassette) (**Figure 2**). To facilitate interpretations, the original films were divided into separate segments for each digest with the marker lanes copied and boxed.

Figure 1. Vector pSIM1278



The vector backbone region, on the left, starts at position 9,957-bp and ends at 19,468-bp (= 9,512-bp). The backbone DNA consists mainly of bacterial DNA and is intended only to support maintenance of the DNA insert prior to plant transformation. The DNA insert region, including flanking Border sequences, is on the right from 19,469-bp to 19,660-bp and from 1-bp to 9,956-bp, which is a total of 10,148-bp. The DNA insert consists of T-DNA border-like sequences and potato genomic DNA which are intended for stable integration into the plant genome.

Table 1. Genetic elements of the DNA Insert of pSIM1278, from Left Border site to Right Border site

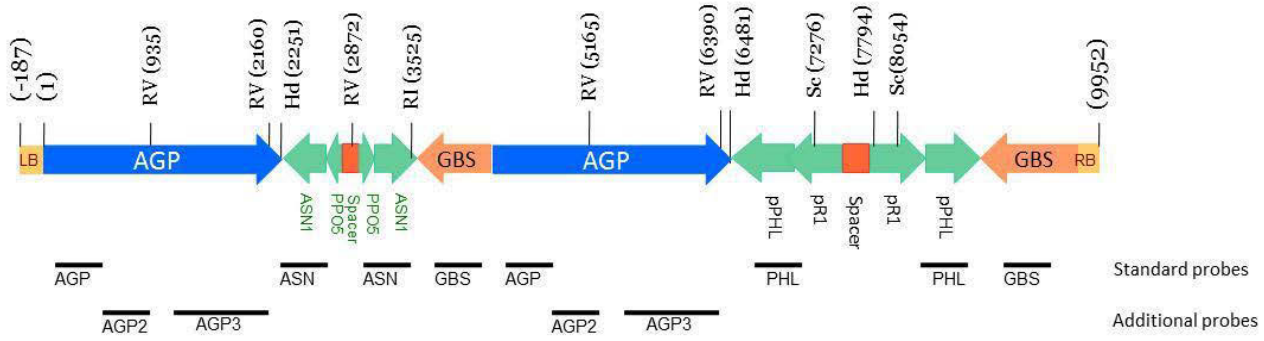
Genetic Element	*Origin*	Intended Function	Genbank Accession Number	Start-End Point in pSIM1278	Reference
1. Left Border (LB) site[1]	Synthetic	Site for secondary cleavage to release single-stranded DNA insert from pSIM1278	AY566555[2] (bases 1-25)	19,469 – 19,493	van Haaren et al. 1989
2. DNA flanking the LB sequence	*S. tuberosum* var. Ranger Russet	Supports secondary cleavage at LB	AY566555[2] (bases 26-187)	19,494 – 19,655	
3. KpnI restriction site	*S. tuberosum*	Site for connection of DNA insert with LB flanking sequence.	AF393847.1	19,656 –1	
4. Promoter for the ADP glucose pyrophosphorylase gene (pAgp), 1st copy	*S. tuberosum* var. Ranger Russet	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of Asn1 and Ppo5, especially in tubers	HM363752	2-2,261	
5. Fragment of the asparagine synthetase-1 (Asn1) gene (1st copy antisense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (9) double stranded RNA that triggers the degradation of Asn1 transcripts to impair asparagine formation	HM363759	2,262-2,666	Chawla et al. 2012[3]
6. 3'-untranslated sequence of the polyphenol oxidase-5 gene (Ppo5) (1st copy, in antisense orientation)	*S. verrucosum*	Generates with (8) double stranded RNA that triggers the degradation of Ppo5 transcripts to block black spot bruise development	HM363754	2,667-2,810	
7. Spacer-1	*S. tuberosum* var. Ranger Russet	Sequence between the 1st inverted repeats	HM363753	2,817-2,973	
8. 3'-untranslated sequence of the polyphenol oxidase-5 gene (Ppo5) (2nd copy, in sense orientation)	*S. verrucosum*	Generates (6) double stranded RNA that triggers the degradation of Ppo5 transcripts to block black spot bruise development	HM363754	2,974-3,117	
9. Fragment of the asparagine synthetase-1 (Asn1) gene (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (5) double stranded RNA that triggers the degradation of Asn1 transcripts to impair asparagine formation	HM363759	3,118-3,523	Chawla et al. 2012[3]
10. 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	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of Asn1 and Ppo5, especially in tubers	HM363755	3,530-4,215	
11. pAgp, 2nd copy	*S. tuberosum* var. Ranger Russet	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	HM363752	4,232-6,491	
12. Fragment of promoter for the potato phosphorylase-L (pPhL) gene (1st copy, in antisense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (16) double stranded RNA that triggers the degradation of PhL transcripts to limit the formation of reducing sugars through starch degradation	HM363758	6,492-7,000	
13. Fragment of promoter for the potato R1 gene (pR1) (1st copy, in antisense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (15) double stranded RNA that triggers the degradation of R1 transcripts to limit the formation of reducing sugars through starch degradation	HM363757	7,001-7,532	
14. Spacer-2	*S. tuberosum* var. Ranger Russet	Sequence between the 2nd inverted repeat	HM363756	7,539-7,796	

Table 1 Continued. Genetic elements of the DNA Insert of pSIM1278, from Left Border site to Right Border site

Genetic Element	Origin	Intended Function	Genbank Accession Number	Start-End Point in pSIM1278	Reference
15. Fragment of promoter for the potato R1 gene (pR1) (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (13) double stranded RNA that triggers the degradation of R1 transcripts to limit the formation of reducing sugars through starch degradation	HM363757	7,797-8,328	
16. Fragment of promoter for the potato phosphorylase-L (pPhL) gene (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (12) double stranded RNA containing this sequence will trigger the degradation of PhL transcript to limit the formation of reducing sugars through starch degradation	HM363758	8,329-8,837	
17. pGbss (2nd copy, convergent orientation relative to the 2nd copy of pAgp)	*S. tuberosum* var. Ranger Russet	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	HM363755	8,838-9,761	
18. SacI restriction site	*S. tuberosum*	Site for connection of DNA insert with RB flanking sequence.	AF143202	9,762 – 9,767	
19. DNA flanking the RB sequence	*S. tuberosum* var. Ranger Russet	Supports primary cleavage at RB-Like site	AY566555[2] (bases 231-391)	9,771 – 9,931	
20. Right Border (RB) sequence[1]	Synthetic	Site for primary cleavage to release single stranded DNA insert from pSIM1278	AY566555[2] (bases 392-416)	9,932 - 9,956	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]GenBank Accession AY566555 was revised to clarify the sources of DNA for the Border regions.
[3]ASN1 described as genetic elements 5 and 9 is referred to as StAst1 in Chawla *et al.* 2012.

Figure 2. DNA Insert Probes



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII
LB = Left Border like region containing 25-bp Left Border and 162-bp flanking sequence.
RB = Right Border like region containing 25-bp Right Border and 161-bp flanking sequence.
Position 1 starts from the first *Kpn*I cutting site in pSIM1278.

Hybridizing bands were based on their approximate sizes and grouped into three different classes (See summary explaining band interpretation in **Table 2**). For clarification purposes, bands in the gels were read by aligning the top with the marker band (see line marking top of band in example here). 

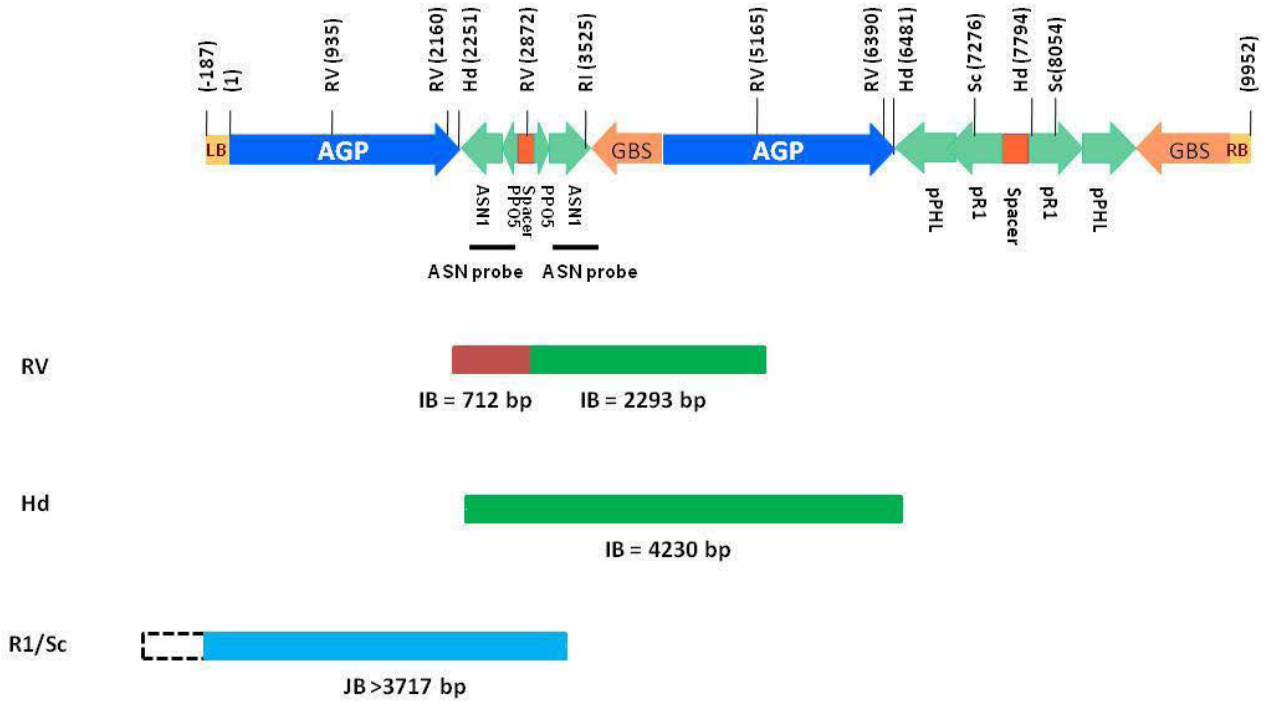
- **Original Bands (OBs)**: Plant sequences that hybridize with a DNA insert probe but are not associated with the inserted DNA. They are indicative of hybridizing sequences that are present in both untransformed and transformed plants (shown as "◀").
- **Internal Bands (IBs)**: Plant sequences that hybridize with a probe, within the inserted DNA. These DNA fragments with predicted sizes are positioned within the DNA insert and indicative of DNA integrity (shown as "◁"). Everything is based on 1 copy of the DNA insert so that the expected numbers are if we have one copy.
- **Junction Bands (JBs)**: Plant sequences that hybridize with a probe and comprise part of both the inserted DNA and the insert-flanking DNA. These sequences comprise both part of the DNA insert and flanking plant DNA, have unique sizes, and are indicative of copy number (shown as "◀").

Observed and Expected Bands: Differences in the number of observed bands (Obs.) compared to expected (Exp.) bands was noted. The data showing more observed bands compared to expected bands shows us that we have more than one copy or an additional partial copy. If the expected band is missing or smaller than the expected size, the integrated DNA insert has a deletion.

In some cases, Southern blots shown in this appendix will use the same probes and enzyme digests as found in other studies (for example, see **Appendix 3. Evidence for Stability of the Inserted DNA**). The observations may differ, depending upon the conditions under which the gels were run. Therefore all bands described in the tables representing Southern blot data in this appendix refer to the gels presented within this appendix.

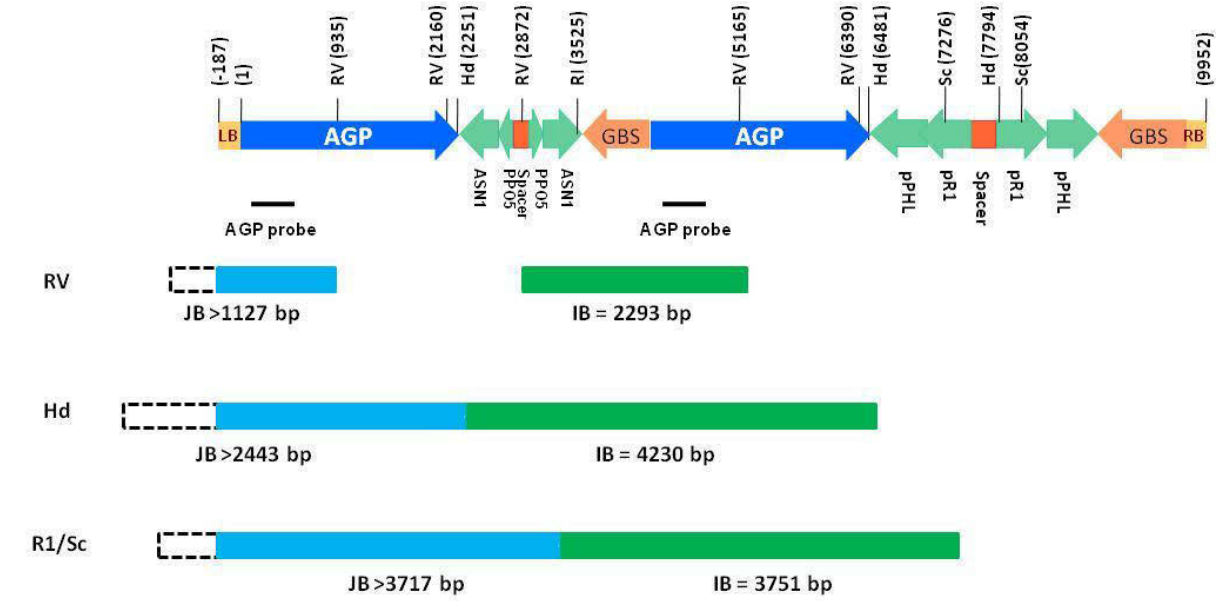
Summary of Gel Evaluation: In **Table 2**, we summarized how gels were interpreted to determine original bands, along with internal and junction bands of the inserted DNA. In addition, in **Figures 3-6** below, we illustrate the restriction sites and where hybridization occurs on the DNA insert for the various probes, thus summarizing how bands are identified through a visual illustration. Also included, is a summary of the expected number of original bands for all the varieties (**Table 3).**

Figure 3. Expected IB and JB of DNA Insert Digested with Various Enzymes and Hybridized with ASN Probe



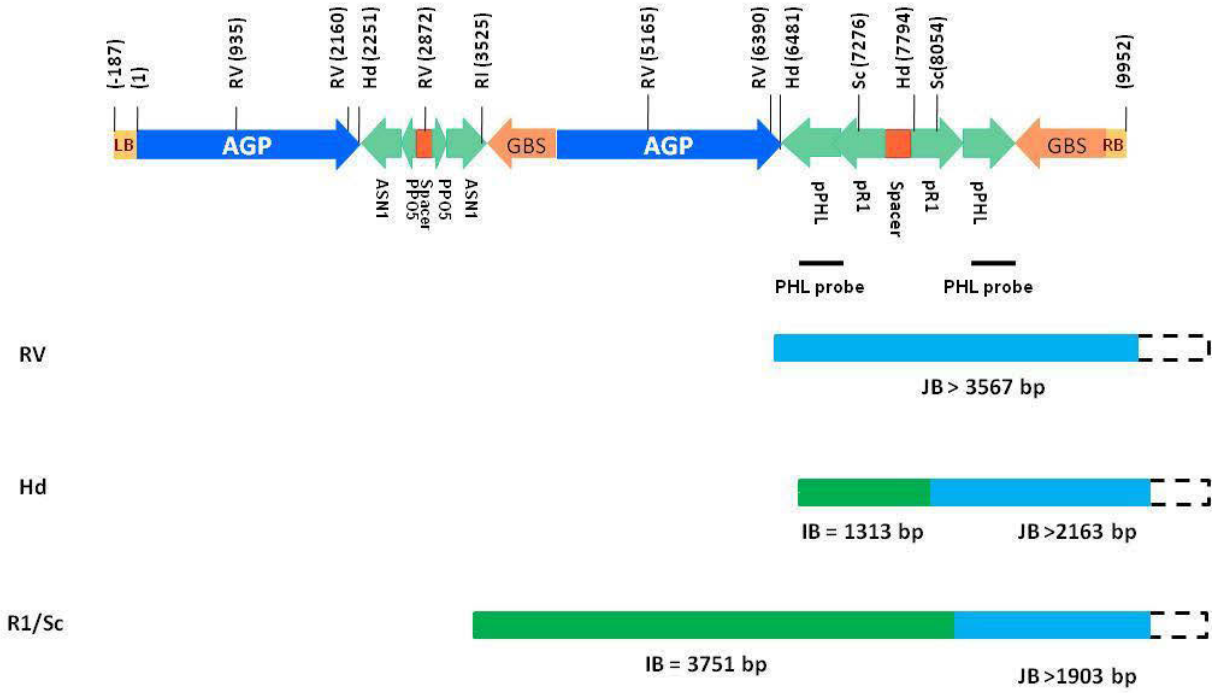
RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII
LB = Left Border like region containing 25-bp Left Border and 162-bp flanking sequence.
RB = Right Border like region containing 25-bp Right Border and 161-bp flanking sequence.

Figure 4. Expected IB and JB of DNA Insert Digested with Various Enzymes and Hybridized with AGP Probe



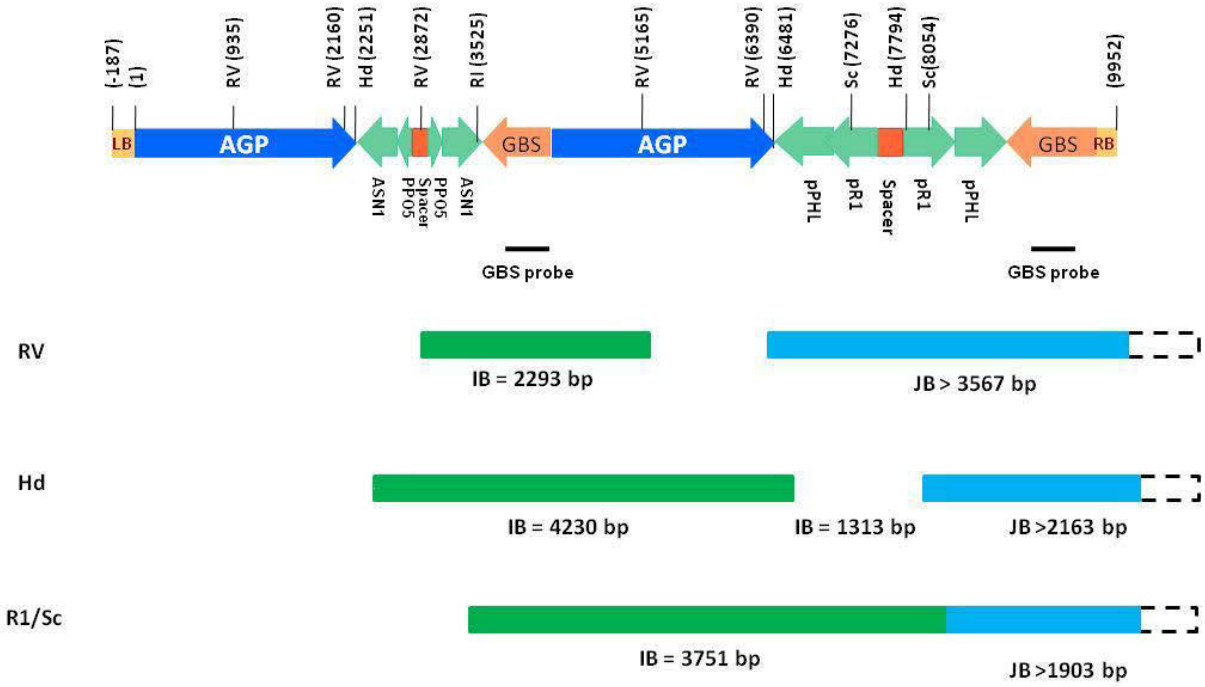
RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII
LB = Left Border like region containing 25-bp Left Border and 162-bp flanking sequence.
RB = Right Border like region containing 25-bp Right Border and 161-bp flanking sequence.

Figure 5. Expected IB and JB of DNA Insert Digested with Various Enzymes and Hybridized with PHL Probe



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII
LB = Left Border like region containing 25-bp Left Border and 162-bp flanking sequence.
RB = Right Border like region containing 25-bp Right Border and 161-bp flanking sequence.

Figure 6. Expected IB and JB of DNA Insert Digested with Various Enzymes and Hybridized with GBS Probe



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII
LB = Left Border like region containing 25-bp Left Border and 162-bp flanking sequence.
RB = Right Border like region containing 25-bp Right Border and 161-bp flanking sequence.

Table 2. Band Interpretation in Events

Probe	Restriction Enzyme	Size (bp)	Position on DNA Insert	Band Type	Expected Bands	Notes:
AGP	EcoRV	Variable	Variable	OB	3-5	Multiple bands relating to variety
		2293	2872-5165	IB	1	Part of PPO/ASN inverted repeat, 1st GBS promoter and left portion of the second AGP promoter
		≥1127	LB Flanking region to AGP 935	JB	1	Indicates at least a portion of the left border side of the DNA insert
AGP	HindIII	Variable	Variable	OB	3-6	Multiple bands relating to variety
		4230	2251-6481	IB	1	PPO/ASN inverted repeat, 1st GBS promoter and left portion of the second AGP promoter
		≥2443	LB flanking region to 2251	JB	1	Indicates at least a portion of the left border side of the DNA insert
AGP	EcoRI/ScaI	Variable	Variable	OB	3-5	Multiple bands relating to variety
		3751	3525-7276	IB	1	Comprising a DNA insert fragment consisting of 1st GBS promoter, 2nd AGP promoter and part of the PHL/R1 inverted repeat
		≥3717	LB region	JB	1	Predicted size indicative of an independent insertion comprising at least the left border side of the DNA insert
ASN	EcoRV	Variable	Variable	OB	1-4	Multiple bands relating to variety
		712, 2293	2160-2872, 2872-5165	IB-1, IB-2	2	Indicative of intact *ASN1*/ *PPO5*-derived inverted repeat and 1st GBS promoter and left portion of the 2nd AGP promoter
ASN	HindIII	Variable	Variable	OB	1-3	Multiple bands relating to variety
		4230	2251-6481	IB	1	Indicative of intact *ASN1*/ *PPO5*-derived inverted repeat as well as 1st GBSs and 2nd AGP promoter

Variable = Size and position of Original bands (OB) are variable depending on potato variety, and position of Junction bands (JB) is variable for each event, depending on the DNA insert integration site.

Table 2 (Continued). Band Interpretation in Events

Probe	Restriction Enzyme	Size (bp)	Position on DNA insert	Band Type	Expected Bands	Notes:
ASN	EcoRI/ScaI	Variable	Variable	OB	2-4	Variable
		≥3717	Variable	JB	1	Indicative of independent insertion comprising at least the left border side of the DNA insert
GBS	EcoRV	Variable	Variable	OB	2-3	Variable
		2293	2872-5165	IB	1	Comprising part of PPO/ASN inverted repeat, 1st GBS promoter and left portion of the second AGP promoter
		≥3567	Variable	JB	1	Indicative of independent insertion comprising at least the right border side of the DNA insert
GBS	HindIII	Variable	Variable	OB	1-2	Variable
		4230	2251-6481	IB	1	Comprising intact *ASN1*/ *PPO5*-derived inverted repeat as well as 1st GBSs and 2nd AGP promoter
		≥2163	Variable	JB	1	Indicative of independent insertion comprising at least the right border side of the DNA insert
GBS	EcoRI/ScaI	Variable	Variable	OB	2-3	Variable
		3751	3525-7276	IB	1	Comprising 1st GBS promoter, 2nd AGP promoter and part of the PHL/R1 inverted repeat
		≥1903	Variable	JB	1	Indicative of independent insertion comprising at least the right border side of the DNA insert
PHL	EcoRV	Variable	Variable	OB	3	Variable
		≥3567	Variable	JB	1	Indicative of independent insertion comprising at least the right border side of the DNA insert
PHL	HindIII	Variable	Variable	OB	3	Variable
		1313	6481-7794	IB	1	Comprising part of the PHL/R1 inverted repeat
		≥2163	Variable	JB	1	Indicative of independent insertion comprising at least the right border side of the DNA insert
PHL	EcoRI/ScaI	Variable	Variable	OB	1-5	Variable
		3751	3525-7276	IB	1	Comprising 1st GBS promoter, 2nd AGP promoter and part of the PHL/R1 inverted repeat
		≥1903	Variable	JB	1	Indicative of independent insertion comprising at least the right border side of the DNA insert

Variable = Size and position of Original bands (OB) are variable depending on potato variety, and position of Junction bands (JB) is variable for each event, depending on the DNA insert integration site.

Table 3. Number of Original Bands (OBs) for Conventional (Wild Type, WT) Varieties

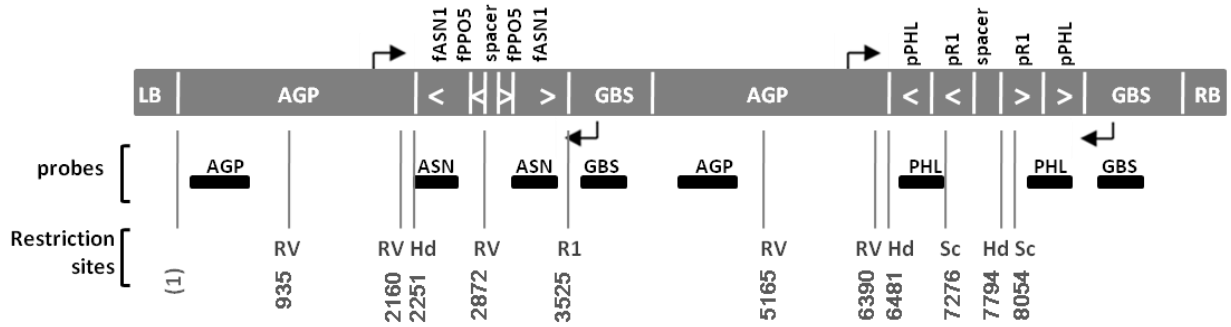
Probe	Restriction Enzyme	Ranger Russet (F)	Russet Burbank (E)	Atlantic (J)	G	H
AGP	EcoRV	3	3	4	4	5
	HindIII	4	4	5	3	6
	EcoR1/Scal	5	3	4	4	5
ASN	EcoRV	4	3	1	2	1
	HindIII	3	3	1	1	1
	EcoR1/Scal	3	4	2	2	2
PHL	EcoRV	3	3	3	3	3
	HindIII	3	3	3	3	3
	EcoR1/Scal	1	2	5	5	3
GBS	EcoRV	3	2	3	2	2
	HindIII	2	1	2	1	1
	EcoR1/Scal	3	2	3	2	2

RESULTS

Copy Number and Integrity: Ranger Russet F10

Plants were molecularly analyzed by Southern blot hybridization using DNA extracted from leaves of greenhouse-grown plants (see **Materials and Methods**). F10 contains a single complete copy of the DNA insert (**Figure 7**). This conclusion was deduced from hybridizations shown in **Figures 8-11** which are summarized in **Table 4**. At the Left Border site, a single **JB** was visualized when *Eco*RV, *Hin*dIII and *Eco*RI/*Sca*I digests were hybridized with the AGP probe. Similarly, hybridization of *Eco*RI/*Sca*I digests with the ASN probe visualized a single **JB**. The presence of a single **JB** at the Right Border side was deduced from hybridizations of *Eco*RV, *Hin*dIII, and *Eco*RI*/Sca*I digests with the GBS and PHL probes.

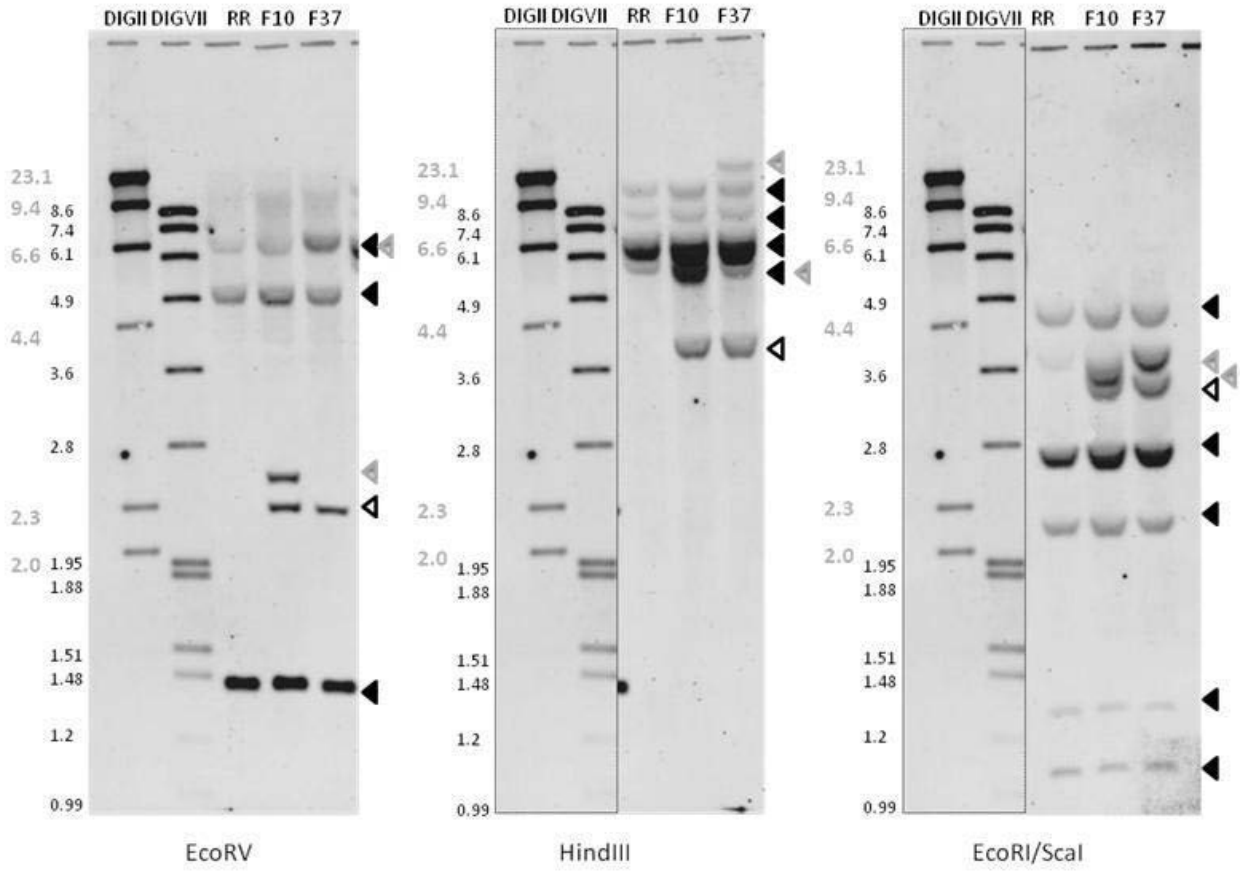
Figure 7. F10 Insert



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII

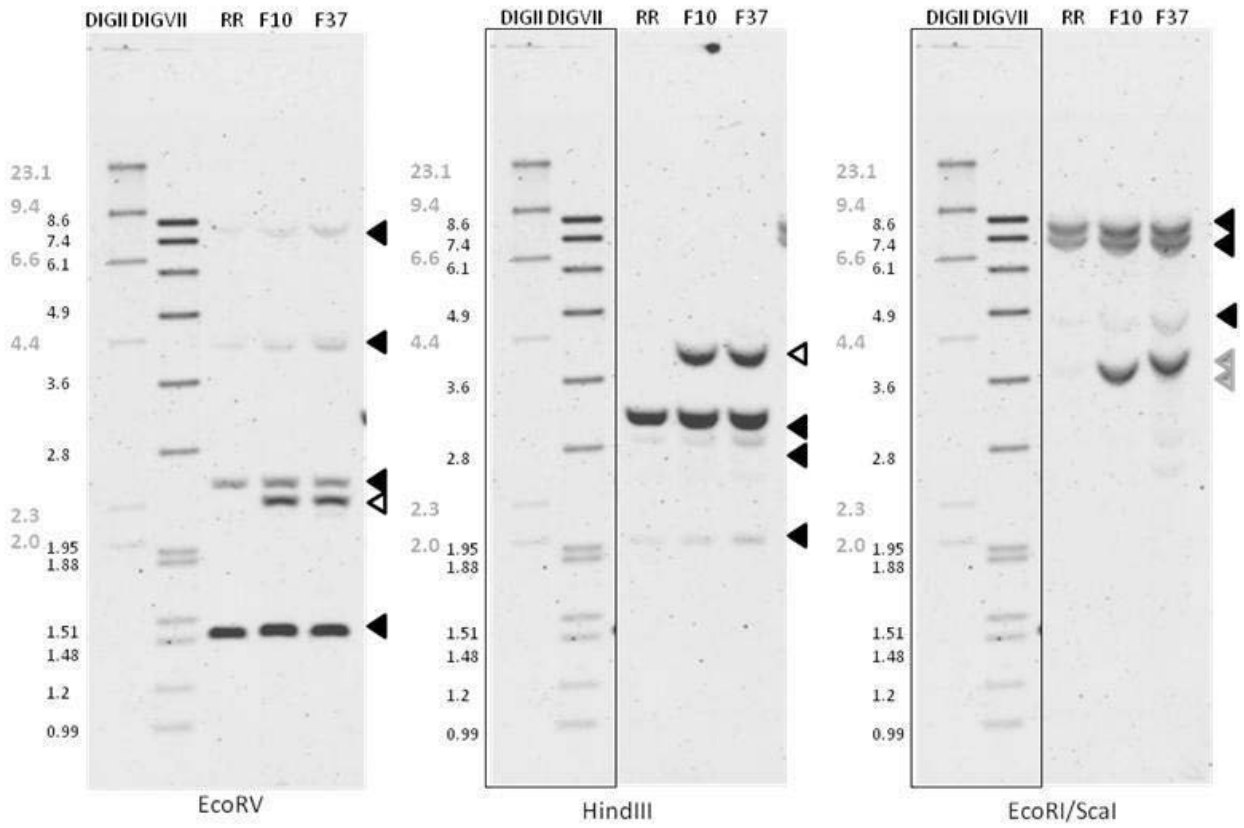
Integrity of the DNA insert is indicated by the predicted sizes of **IBs** visualized in (a) all three digests hybridized with the AGP probe, (b) all three digests hybridized with the GBS probe, (c) *Eco*RV and *Hin*dIII digests with the ASN probe, and (d) *Hin*dIII and *Eco*RI/*Sca*I digests hybridized with the PHL probe. Furthermore, **JBs** are larger than the minimum predicted size from internal restriction site to cleavage site in the left border (>1.1-kb) as visualized with *Eco*RV digest hybridized with the AGP probe, and in the right border (>1.9-kb) with *Eco*RI*/ScaI* digests hybridized with the GBS probe. Moreover, no other band indicative of any truncation was observed indicating that the integrated copy was complete. Intactness was confirmed by experiments shown in **Appendix 4. Junction Analysis and Event-Specific Detection**.

Figure 8. Ranger Russet DNA Hybridization with the AGP Probe



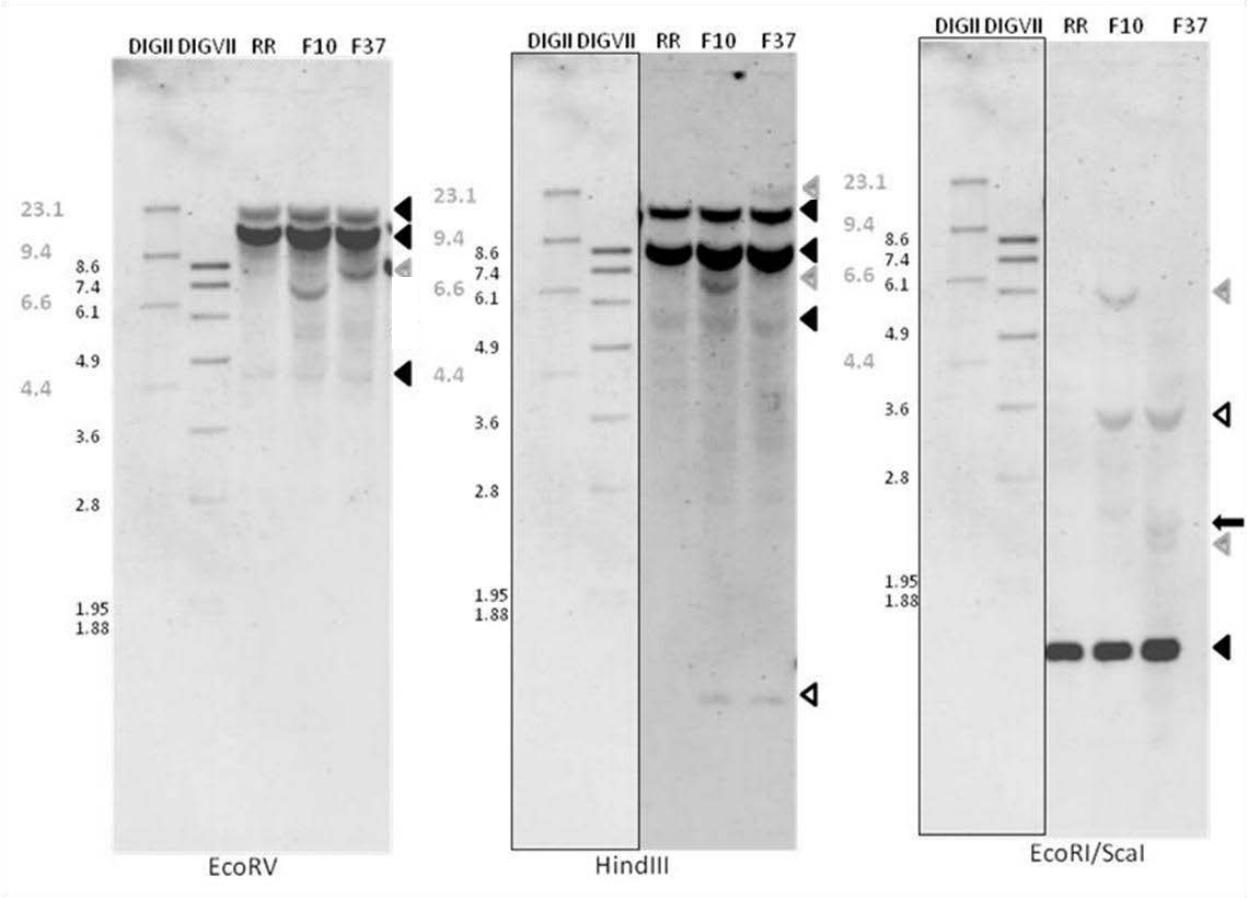
Genomic DNA of Ranger Russet control (RR) and events F10 and F37 were digested and hybridized with the AGP probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 9. Ranger Russet DNA Hybridization with the ASN Probe



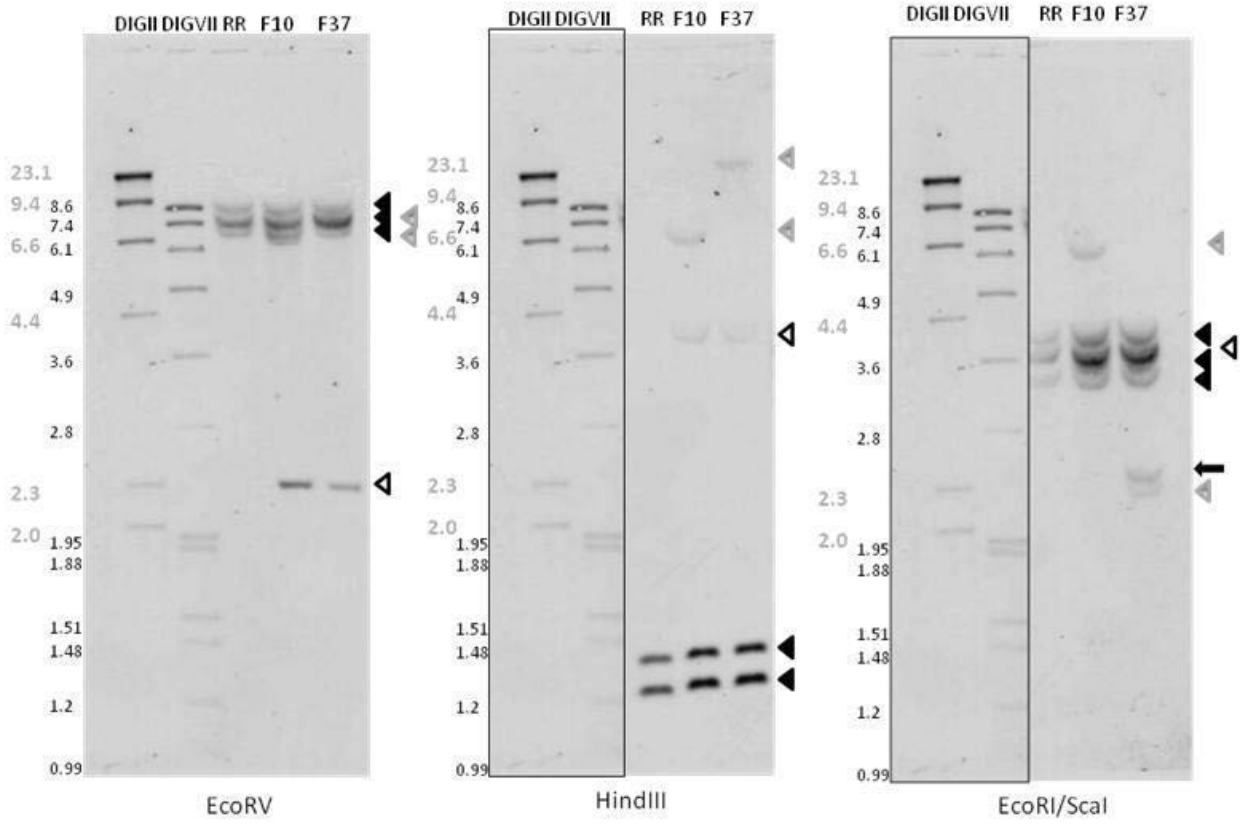
Genomic DNA of Ranger Russet control (RR) and events F10 and F37 were digested and hybridized with the ASN probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 10. Ranger Russet DNA Hybridization with the PHL Probe



Genomic DNA of Ranger Russet control (RR) and events F10 and F37 were digested and hybridized with the PHL probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**. Partial digestion of *Eco*RI site due to overlapping DNA methylation is marked by an arrow.

Figure 11. Ranger Russet DNA Hybridization with the GBS Probe



Genomic DNA of Ranger Russet control (RR) and events F10 and F37 were digested and hybridized with the GBS probe. The enzymes used are indicated below each panel. Size of the DigII and Dig VII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**. Partial digestion of *Eco*RI site due to overlapping DNA methylation is marked by an arrow.

Table 4. Ranger Russet Event F10: DNA Fragments Hybridizing with DNA Insert -Derived Probes
(Summary of observations from **Figures 8-11**)

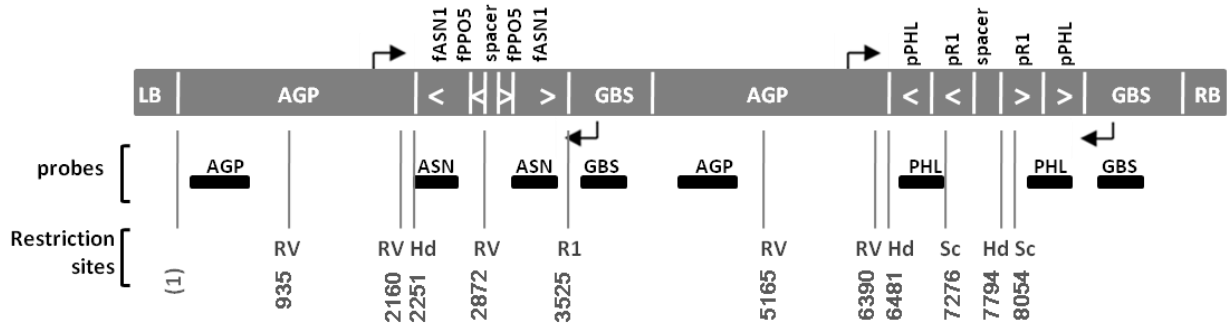
Probe	Restriction Enzyme	Type of Band					Interpretation
		OB	**IB Exp.**	**IB Obs.**	**JB Exp.**	**JB Obs.**	
AGP Figure 8	EcoRV	6.7 5.0 1.4	2.3	2.3	1.1+	2.6	**1.** ***Copy number at Left Border side*** Visualization of a single **JB** in AGP and ASN digests indicates that F10 contains a single DNA insert (at the Left Border side).
	HindIII	16 8.0 6.5 5.8	4.2	4.2	2.4+	5.7	**2.** ***Copy number at Right Border side*** Visualization of a single **JB** in PHL and GBS digests indicates that F10 contains a single DNA insert (at the Right Border side).
	EcoRI/ScaI	4.8 2.7 2.2 1.3 1.0	3.8	3.6	3.7+	3.8	**3.** ***Internal DNA insert Integrity*** Integrity of the DNA insert is indicated by the fact that the **IBs** visualized in all DNA digests have nearly the expected sizes (see note 5 below regarding variations). See also Figure 40 for 0.7-kb IB.
ASN Figure 9	EcoRV	8.6 4.4 2.5 1.5	2.3, 0.7	2.3, 0.7#	none	none	**4.** ***DNA insert Integrity at the extremities*** The DNA insert is complete because all JBs are greater than the minimum size expected. Closest to JBs are *EcoRV* fragments with AGP probe (LB) and *EcoRI/ScaI* fragments with GBS probe (RB).
	HindIII	3.2 2.9 2.0	4.2	4.2	none	none	
	EcoRI/ScaI	8.2 7.3 4.8	none	none	3.7+	3.9	**5.** ***Variation between expected and observed sizes in DNA insert.*** Slight differences were found between the observed and expected size of a 3.8-kb internal band between restriction sites for EcoR1 at 3,525-bp and Sca1 at 7,276-bp. This band was expected with the AGP, PHL,and GBS probes with EcoR1 and Sca1, but appeared sometimes at 3.6-kb. This may be because of differences in gel loading or possibly the influence of plant material resulted in faster movement within the gel. See further discussion in section titled "Additional Studies on DNA insert Integrity for all Events."
PHL Figure 10	EcoRV	22 20 4.7	none	none	3.6+	7.2	
	HindIII	20 8.6 6.0	1.3	1.3	2.2+	7.0	
	EcoRI/ScaI	1.5	3.8	3.6	1.9+	6.1	
GBS Figure 11	EcoRV	8.6 7.8 7.2	2.3	2.3	3.6+	6.6	
	HindIII	1.4 1.3	4.2	4.2	2.2+	6.6	
	EcoRI/ScaI	4.0 3.7 3.4	3.8	3.8	1.9+	6.1	

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Copy Number and Integrity: Ranger Russet F37

F37 contains a single complete copy of the DNA insert (see **Figure 12. F37 Insert**). This conclusion was deduced from hybridizations shown in **Figures 8-11** which are summarized in **Table 5**. The following hybridizations indicated predicted sizes for **IBs**: *Eco*RV*, Hin*dIII *and EcoRI/ScaI* digests hybridized with the AGP and the GBS probes, *Eco*RV, *Hin*dIII digests hybridized with the ASN probe and *Hin*dIII, *Eco*RI*/Sca*I I digests hybridized with the PHL probe. Integrity of the DNA insert is indicated by the fact that the **IBs** visualized in various DNA digests and probes have predicted sizes.

Figure 12. F37 Insert



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII

Furthermore, the following hybridizations indicate that **JBs** are larger than the minimum predicted size from internal restriction site to cleavage site in the border: *Eco*RV, *Hin*dIII, and *Eco*RI*/Sca*I digests hybridized with the AGP probe, *Eco*RI*/ Sca*I digests hybridized with the ASN probe; *Eco*RV, *Hin*dIII, and *Eco*RI*/Sca*I digests hybridized with the PHL and GBS probe. For instance, the **JB** visualized with the AGP probe in *Eco*RV digest is larger than the minimum predicted size from restriction site to cleavage site in the left border (>1.1-kb) and GBS probe in *EcoR*I/*Sca*I digest is larger than the minimum predicated size (>1.9–kb) in the right border. Moreover, no other band indicative of any truncation was observed indicating that the integrated copy was complete.

Data were confirmed by sequencing one of the junction fragments shown in **Appendix 4. Junction Analysis and Event-Specific Detection**.

Table 5. Ranger Russet Event F37: DNA Fragments Hybridizing with DNA Insert -Derived Probes
(Summary of observations from **Figures 8-11**)

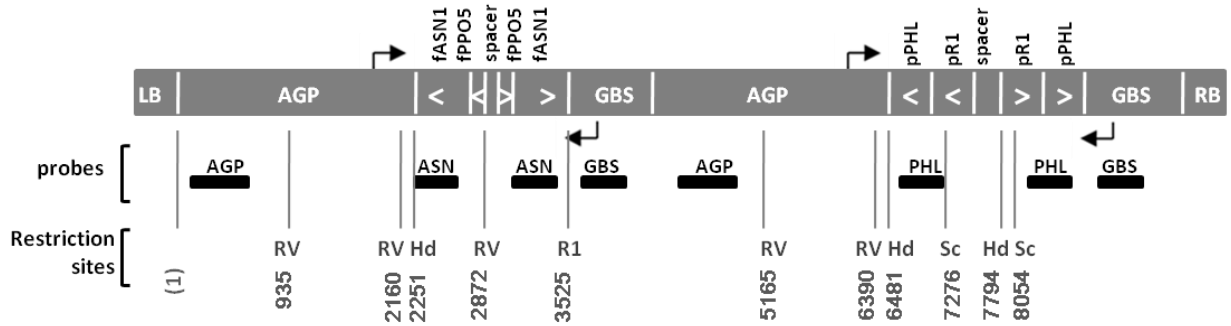
Probe	Restriction Enzyme	Type of Band					Interpretation
		OB	**IB Exp**	**IB Obs**	**JB Exp**	**JB obs**	
AGP Figure 8	EcoRV	6.7 5.0 1.4	2.3	2.3	1.1+	6.7 (double intensity)	**1.** ***Copy number at Left Border side*** Visualization of a single **JB** in AGP and ASN digests indicates that F37 contains a single DNA insert (at least the Left Border side). The double intensity band at 6.7 indicates 1 OB and 1 JB are the same size. **2.** ***Copy number at Right Border side*** Visualization of a single **JB** in most PHL and GBS digests indicate that F37 contains a single DNA insert (at least the Right Border side). **3.** ***Internal DNA insert Integrity*** Integrity of the DNA insert is indicated by the fact that the **IBs** visualized in various DNA digests have nearly the expected sizes (see note 5 below regarding variation). See also Figure 40 for 0.7-kb IB. **4.** ***DNA insert Integrity at the extremities*** The DNA insert is complete because all JBs are greater than the minimum size expected. Closest to JBs are *EcoRV* fragments with AGP probe (LB) and *EcoRI/ScaI* fragments with GBS probe (RB). **5.** ***Variation between expected and observed sizes in DNA insert.*** Slight differences were found between the observed and expected size of a 3.8-kb internal band between restriction sites for EcoR1 at 3,525-bp and Sca1 at 7,276-bp. This band was expected with the AGP, PHL,and GBS probes with EcoR1 and Sca1, but appeared sometimes at 3.6-kb. This may be because of differences in gel loading or possibly the influence of plant material resulted in faster movement within the gel. See further discussion in section titled "Additional Studies on DNA insert Integrity for all Events." *Partial digestion of *Eco*RI site due to overlapping methylation marked by arrow (Figures 10, 11).
	HindIII	16 8.0 6.5 5.8	4.2	4.2	2.4+	>23	
	EcoRI/ScaI	4.8 2.7 2.2 1.3 1.0	3.8	3.6	3.7+	4.0	
ASN Figure 9	EcoRV	8.6 4.4 2.5 1.5	2.3, 0.7	2.3, 0.7#	none	none	
	HindIII	3.2 2.9 2.0	4.2	4.2	none	none	
	EcoRI/ScaI	8.2 7.3 4.8	none	none	3.7+	4.2	
PHL Figure 10	EcoRV	22 20 4.7	none	none	3.6+	8.2	
	HindIII	20 8.6 6.0	1.3	1.3	2.2+	>23	
	EcoRI/ScaI	1.5	3.8	3.6	1.9+	2.4, 2.3*	
GBS Figure 11	EcoRV	8.6 7.8 7.2	2.3	2.3	3.6+	7.7	
	HindIII	1.4 1.3	4.2	4.2	2. 2+	>23	
	EcoRI/ScaI	4.0 3.7 3.4	3.8	3.8	1.9+	2.4, 2.3*	

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Copy Number and Integrity: Russet Burbank E12

E12 contains a single complete copy of the DNA insert (see **Figure 13**). This conclusion was deduced from the hybridizations shown in **Figures 14-17** which are summarized in **Table 6**.

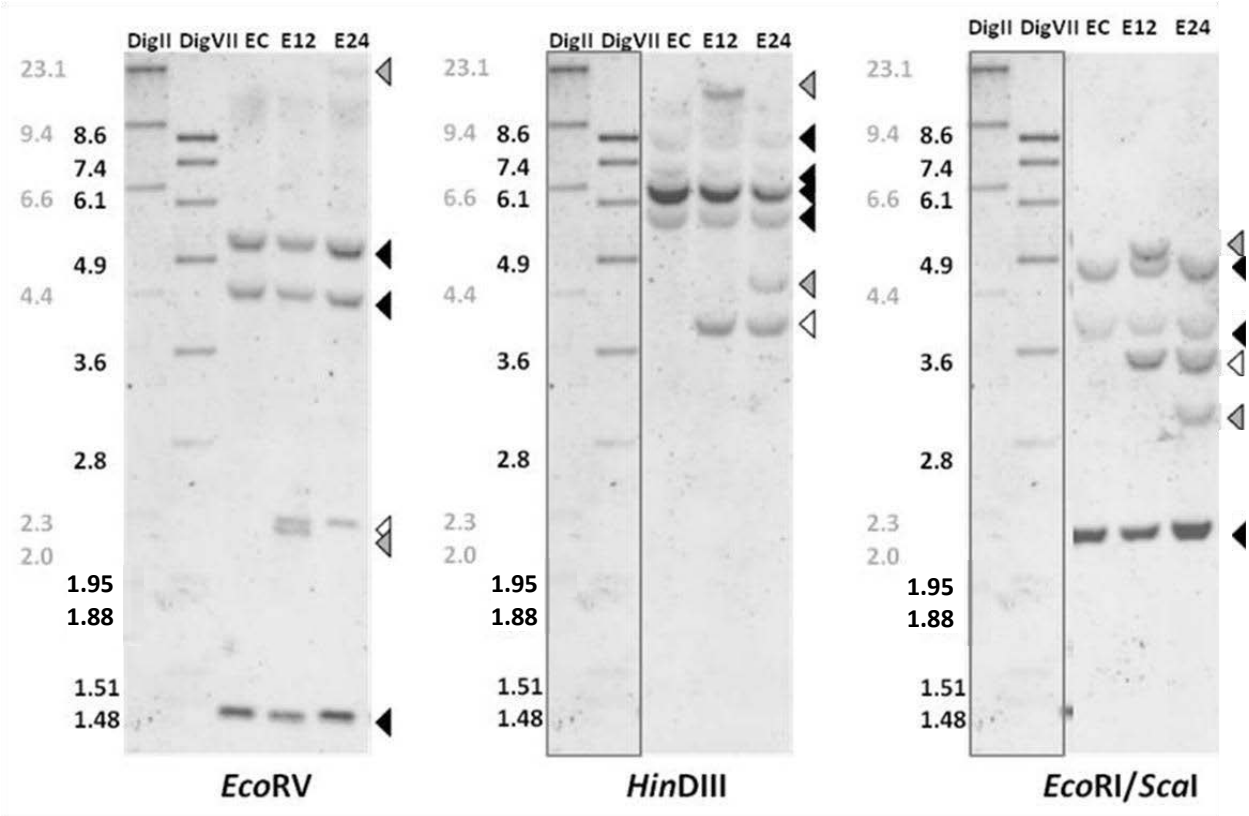
Figure 13. E12 Insert



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII

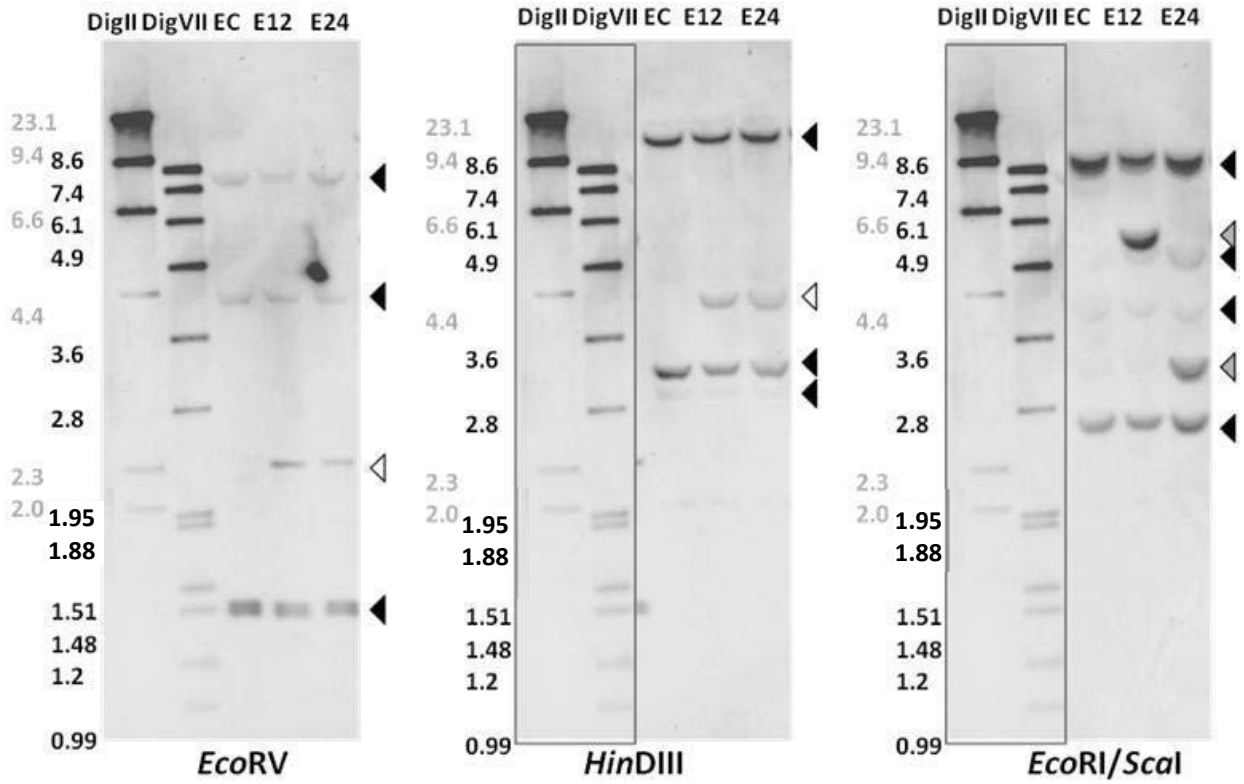
DNA insert copy number assessment was based on the fact that single **JBs** with sizes greater than the minimum predicted sizes (from restriction site to border cleavage site) were visualized for both the Right Border side and the Left Border side when the various DNA digests were hybridized with AGP, ASN, PHL and GBS probes. Intactness of the DNA insert was confirmed by visualization of **IBs** with predicted sizes. This is also confirmed by the junction study, which shows both ends of the DNA insert are intact (**Appendix 4. Junction Analysis and Event-Specific Detection)**.

Figure 14. Russet Burbank DNA Hybridization with the AGP Probe



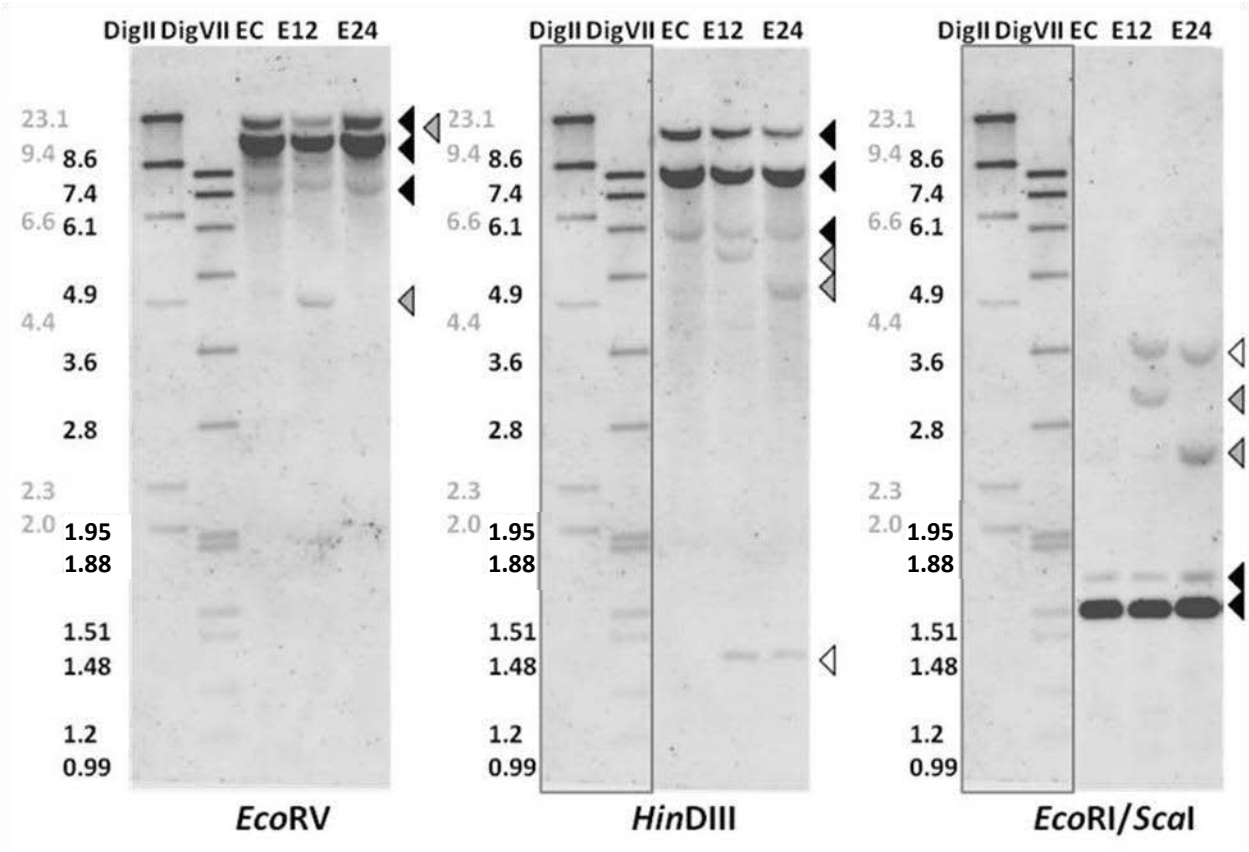
Genomic DNA of Russet Burbank control (EC) and events E12 and E24 were digested and hybridized with the AGP probe. The enzymes used are indicated below each panel. Size of the DigII and Dig VII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**. Partial digestion of *Eco*RI site due to overlapping DNA methylation is marked by an arrow.

Figure 15. Russet Burbank DNA Hybridization with the ASN Probe



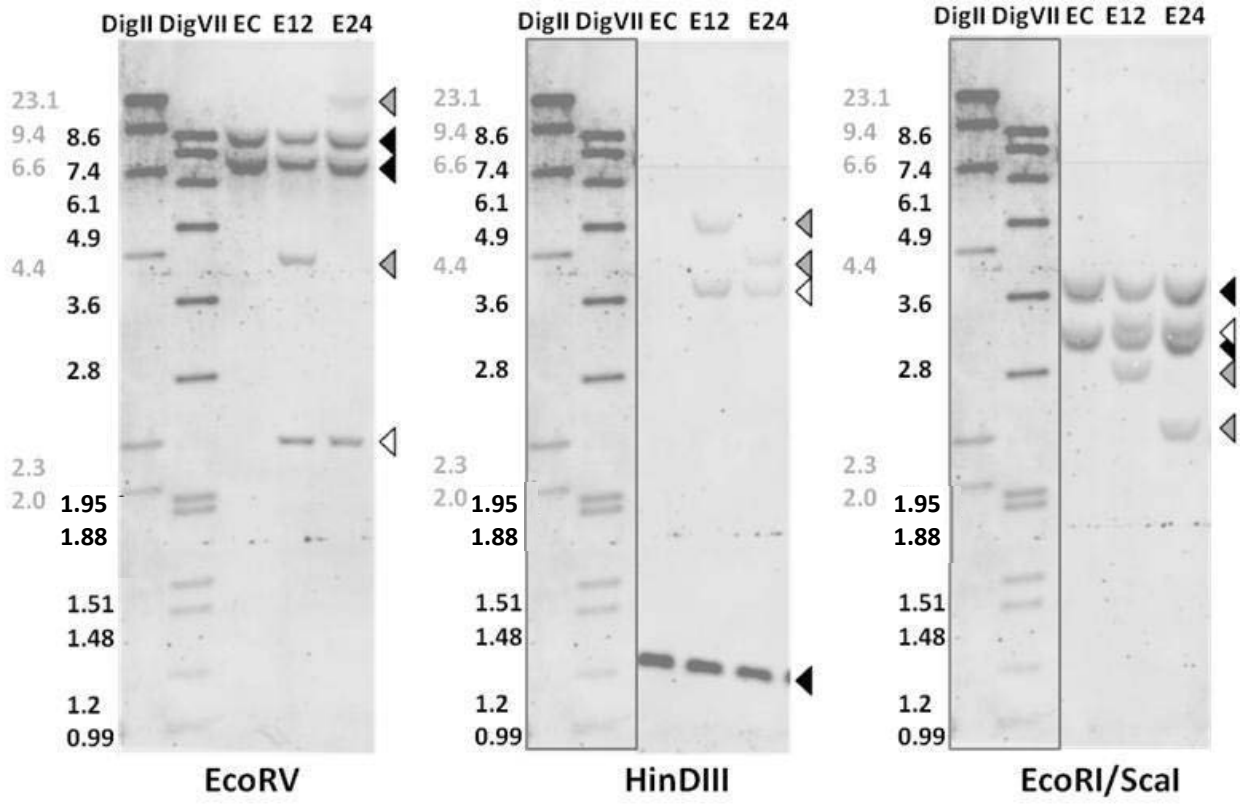
Genomic DNA of Russet Burbank control (EC) and events E12 and E24 were digested and hybridized with the ASN probe. The enzymes used are indicated below each panel. Size of the DigII and Dig VII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 16. Russet Burbank DNA Hybridization with the PHL Probe



Genomic DNA of Russet Burbank control (EC) and events E12 and E24 were digested and hybridized with the PHL probe. The enzymes used are indicated below each panel. Size of the DigII and Dig VII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 17. Russet Burbank DNA Hybridization with the GBS Probe



Genomic DNA of Russet Burbank control (EC) and events E12 and E24 were digested and hybridized with the GBS probe. The enzymes used are indicated below each panel. Size of the DigII and Dig VII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Table 6. Russet Burbank Event E12: DNA Fragments Hybridizing with DNA Insert -Derived Probes
(Summary of observations from **Figures 14-17**)

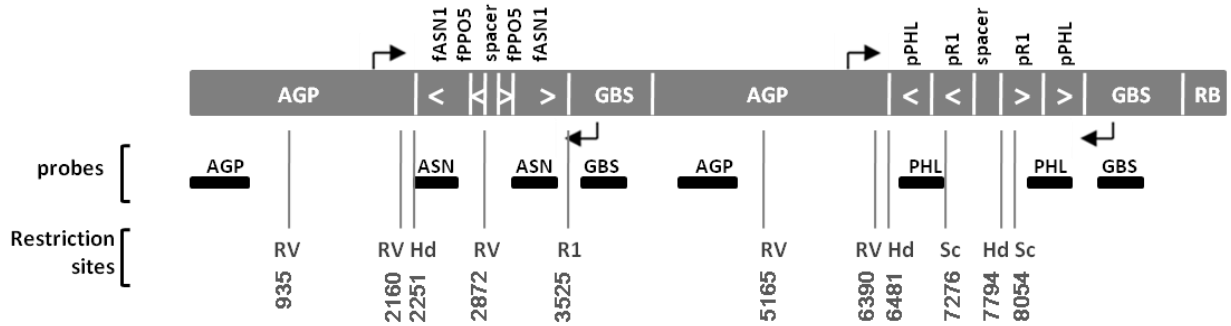
Probe	Restriction Enzyme	Type of Band (kb)					Interpretation
		OB	**IB**	**IB**	**JB**	**JB**	
			Exp.	**Obs.**	**Exp.**	**Obs.**	
AGP Figure 14	EcoRV	5.2 4.4 1.5	2.3	2.3	1.1+	2.2	**1.** ***Copy number at Left Border side*** Visualization of a single **JB** with AGP and ASN probes indicates that E12 contains a single DNA insert (at least the Left Border side). **2.** ***Copy number at Right Border side*** Visualization of a single **JB** with PHL and GBS probes indicates that E12 contains a single DNA insert (at least the Right Border side). **3.** ***Internal DNA insert Integrity*** Integrity of the DNA insert is indicated by the fact that the **IBs** visualized in various DNA digests have nearly the expected sizes (see note 5 below regarding variation). See also Figure 40 for 0.7-kb IB. **4.** ***DNA insert Integrity at the extremities*** No unexpected bands were observed, and all **JBs** have sizes greater than minimum predicted sizes, indicating the overall intactness of the DNA insert. Closest to JBs are *EcoRV* fragments with AGP probe (LB) and *EcoRI/ScaI* fragments with GBS probe (RB). **5.** ***Variation between expected and observed sizes in DNA insert*** Slight differences were found between the observed and expected size of a 3.8-kb internal band between restriction sites for EcoR1 at 3525-bp and Sca1 at 7276-bp. This band was expected with the AGP, PHL,and GBS probes with EcoR1 and Sca1, but appeared sometimes at 3.4 to 3.6-kb. This may be because of differences in gel loading or possibly the influence of plant material resulted in faster movement within the gel. See further discussion in section titled "Additional Studies on DNA insert Integrity for all Events."
	HindIII	8.4 7.0 6.6 5.8	4.2	4.2	2.4+	15	
	EcoRI/ScaI	4.8 4.0 2.3	3.8	3.6	3.7+	5.3	
ASN Figure 15	EcoRV	8.0 4.4 1.5	2.3 0.7	2.3 0.7#	none	none	
	HindIII	12 3.2 3.0	4.2	4.2	none	none	
	EcoRI/ScaI	9.4 4.9 4.0 2.7	none	none	3.7+	5.8	
PHL Figure 16	EcoRV	20.0 15.0 8.0	none	none	3.6+	4.5	
	HindIII	18.0 8.8 6.0	1.3	1.3	2.2+	5.5	
	EcoRI/ScaI	1.6 1.5	3.8	3.6	1.9+	3.3	
GBS Figure 17	EcoRV	8.6 7.6	2.3	2.3	3.6+	4.5	
	HindIII	1.3	4.2	4.2	2.2+	5.2	
	EcoRI/ScaI	4.2 3.2	3.8	3.4	1.9+	3.0	

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Copy Number and Integrity: Russet Burbank E24

E24 contains a single, mostly complete, copy of the DNA insert (see **Figure 18**). This conclusion was deduced from the hybridizations shown in **Figures 14-17** which are summarized in **Table 7**.

Figure 18. E24 Insert



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII

DNA insert copy number assessment was based on the fact that single **JBs** were visualized for both the Right Border side and the Left Border side when the various DNA digests were hybridized with AGP, ASN, PHL and GBS probes.

Visualization of **IBs** with predicted sizes confirmed that the DNA insert was generally intact. However, the DNA insert has an approximate 500-bp deletion at its Left Border side because the associated **JBs** (3.2–kb) are smaller than the minimum size expected (3.7-kb) when AGP and ASN probes were used for hybridization with *Eco*RI/ScaI digests. Since the AGP probe, which starts from the 5' end of AGP promoter, still detected the left side junction signal with lighter intensity, the deletion is within the AGP probe and does not affect efficacy of the silencing constructs. The Right Border junction was sequenced as described in **Appendix 4. Junction Analysis and Event-Specific Detection**.

Table 7. Russet Burbank Event E24: DNA Fragments Hybridizing with DNA Insert -Derived Probes
(Summary of observations from **Figures 14-17**)

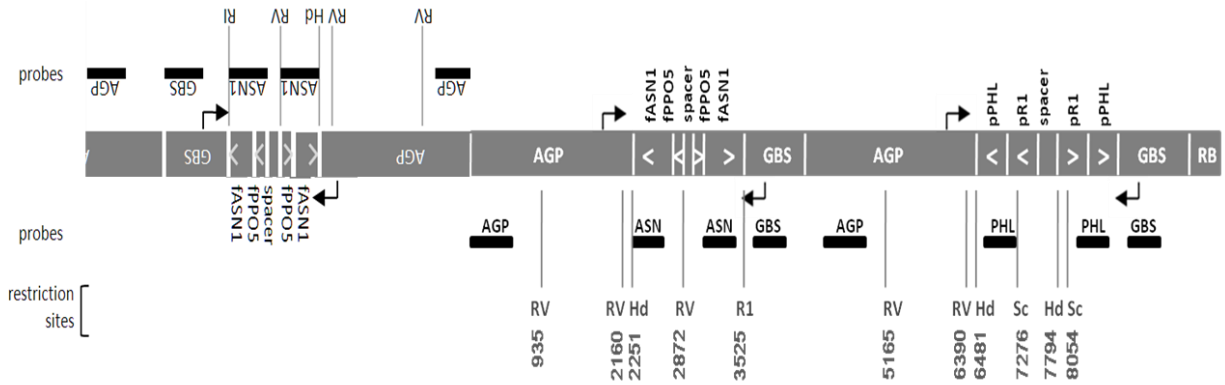
Probe	Restriction Enzyme	Type of Band (kb)					Interpretation
		OB	**IB**	**IB**	**JB**	**JB**	
			Exp.	**Obs.**	**Exp.**	**Obs.**	
AGP Figure 14	EcoRV	5.2 4.4 1.5	2.3	2.3	1.1+	20.0	**1.** ***Copy number at Left Border side*** Visualization of a single **JB** with AGP and ASN probes indicates that E24 contains a single DNA insert (at least Left Border side). **2.** ***Copy number at Right Border side*** Visualization of a single **JB** with PHL and GBS probes indicates that E24 contains a single DNA insert (at least the Right Border side). **3.** ***Internal DNA insert Integrity*** Integrity of the DNA insert is indicated by the fact that the **IBs** visualized in various DNA digests have nearly the expected sizes (see note 5 below regarding variation). See also Figure 40 for 0.7-kb IB. **4.** ***DNA insert Integrity at the extremities*** The DNA insert suffers a small deletion at its Left Border extremity because the **JBs** are smaller than the minimum size expected (> 3.7-kb). The deletion is upstream from where the AGP probe anneals, which means not greater than a few hundred base pairs, and does not affect the functional activity of the *ASN1*/*PPO5* gene silencing cassette. The right side of the DNA insert is intact because all **JBs** of PHL and GBS are greater than the minimum size expected. Closest to JBs are *EcoRV* fragments with AGP probe (LB) and *EcoRI/ScaI* fragments with GBS probe (RB). **5.** ***Variation between expected and observed sizes in DNA insert.*** Slight differences were found between the observed and expected size of a 3.8-kb internal band between restriction sites for EcoR1 at 3525-bp and Sca1 at 7276-bp. This band was expected with the AGP, PHL,and GBS probes with EcoR1 and Sca1, but appeared sometimes at 3.4 to 3.6-kb. This may be because of differences in gel loading or possibly the influence of plant material resulted in faster movement within the gel. See further discussion in section titled "Additional Studies on DNA insert Integrity for all Events."
	HindIII	8.4 7.0 6.6 5.8	4.2	4.2	2.4+	4.6	
	EcoRI/ScaI	4.8 4.0 2.3	3.8	3.6	3.7+	3.2	
ASN Figure 15	EcoRV	8.0 4.4 1.5	2.3 0.7	2.3 0.7#	none	none	
	HindIII	12 3.2 3.0	4.2	4.2	none	none	
	EcoRI/ScaI	9.4 4.9 4.0 2.7	none	none	3.7+	3.2	
PHL Figure 16	EcoRV	20.0 15.0 8.0	none	none	3.6+	23.0	
	HindIII	18.0 8.8 6.0	1.3	1.3	2.2+	4.7	
	EcoRI/ScaI	1.6 1.5	3.8	3.6	1.9+	2.5	
GBS Figure 17	EcoRV	8.6 7.6	2.3	2.3	3.6+	23.0	
	HindIII	1.3	4.2	4.2	2.2+	4.5	
	EcoRI/ScaI	4.2 3.2	3.8	3.4	1.9+	2.5	

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Copy Number and Integrity: Atlantic Event J3

J3 contains one nearly intact and one partial copy of the DNA insert, connected reversely at the left border side, with deletions of LB regions and parts of the adjacent AGP promoters. The partial copy lacks the 3' end of the central AGP and the rest of the R1/PHL promoter silencing cassette (see **Figure 19**). This conclusion was deduced from Southern hybridizations shown in **Figures 20-23** which are summarized in **Table 8**.

Figure 19. J3 Insert

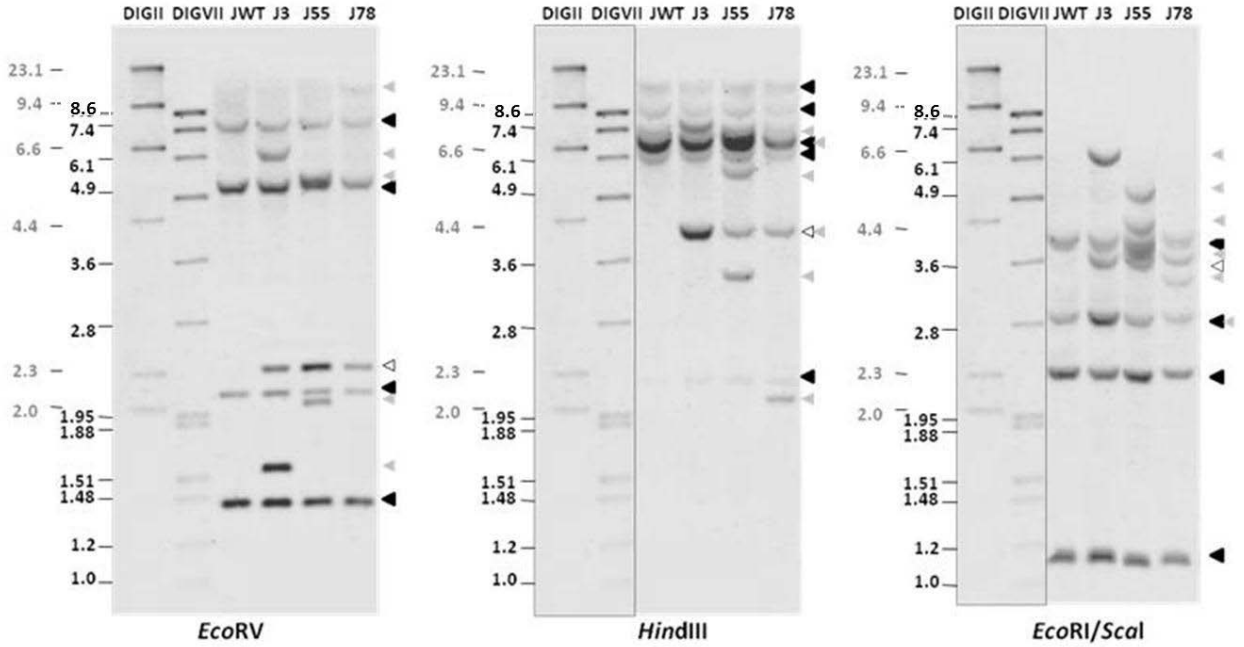


RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII

DNA insert copy number assessment was based on the fact that 2 **JBs** were visualized with hybridizations for the Left Border side. However, only one **IB** was observed when the digested DNA was hybridized with the AGP probe, indicating that a truncation occurred within the central AGP promoter in one copy of the DNA insert. Additional evidence for the occurrence of a truncation comes from the fact that only one **IB** and one **JB** were visualized from DNA digests hybridized with the PHL probe**,** which means that the truncated DNA insert lacks PHL fragments. Furthermore, only one **IB** and two **JBs** rather than two **IBs** and two **JBs** were visualized with the GBS probe (one **IB** and one **JB** per intact DNA insert). One 1.6-kb *Eco*RV band and one 4.2-kb *Hin*dIII band visualized in the hybridization with AGP probe are double intense, suggesting that two copies of the DNA insert can be connected head to head with deletion of Left Borders and part of AGP promoters as shown in **Figure 19** (J3 Insert). Indeed, 6.6-kb bands containing AGP or ASN from both DNA insert copies are found in the EcoR1/ScaI DNA digestions probed with AGP and ASN, respectively.

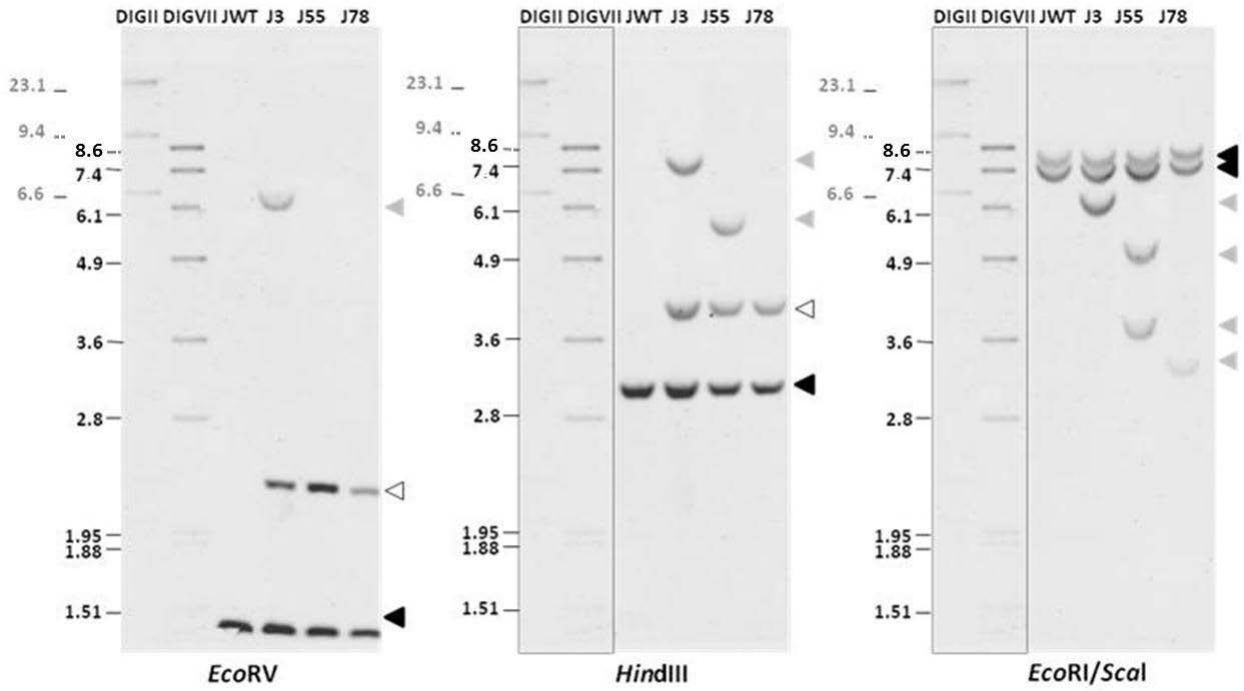
This structure comprising two connected DNA inserts was also confirmed by sequence analysis described in **Appendix 4. Junction Analysis and Event-Specific Detection**.

Figure 20. Atlantic Hybridization with the AGP Probe



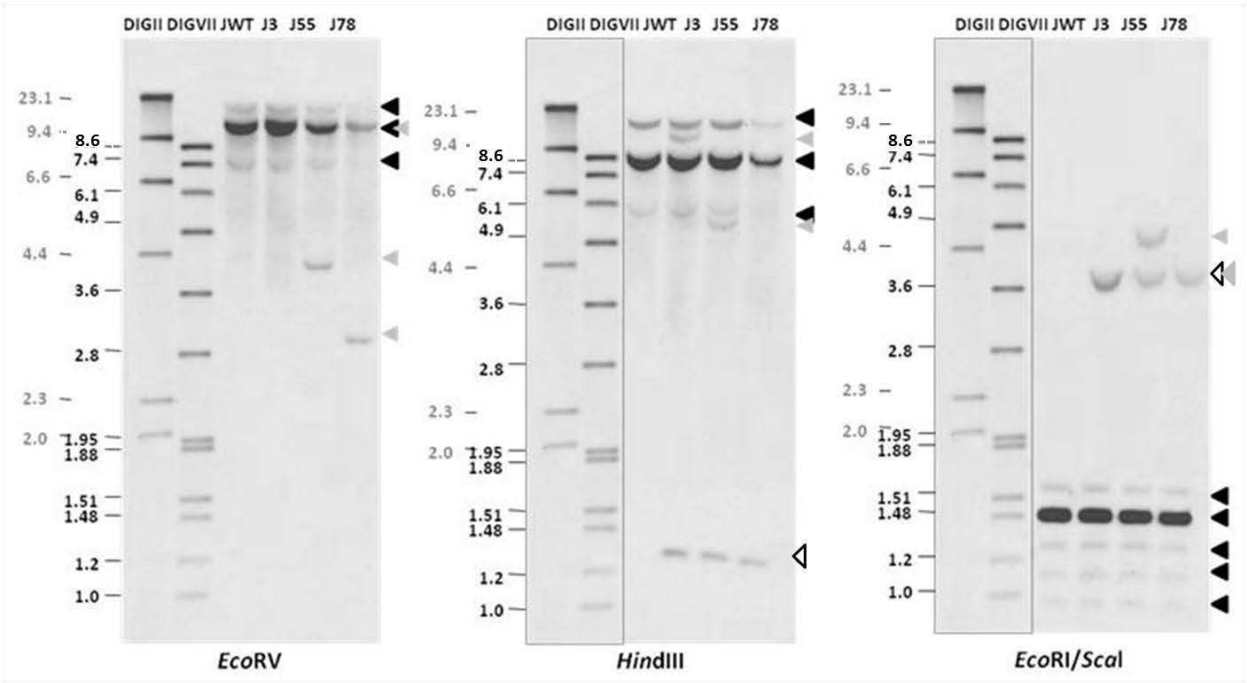
Genomic DNA of Atlantic control (JC) and events J3, J55 and J78 were digested and hybridized with the AGP probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 21. Atlantic Hybridization with the ASN Probe



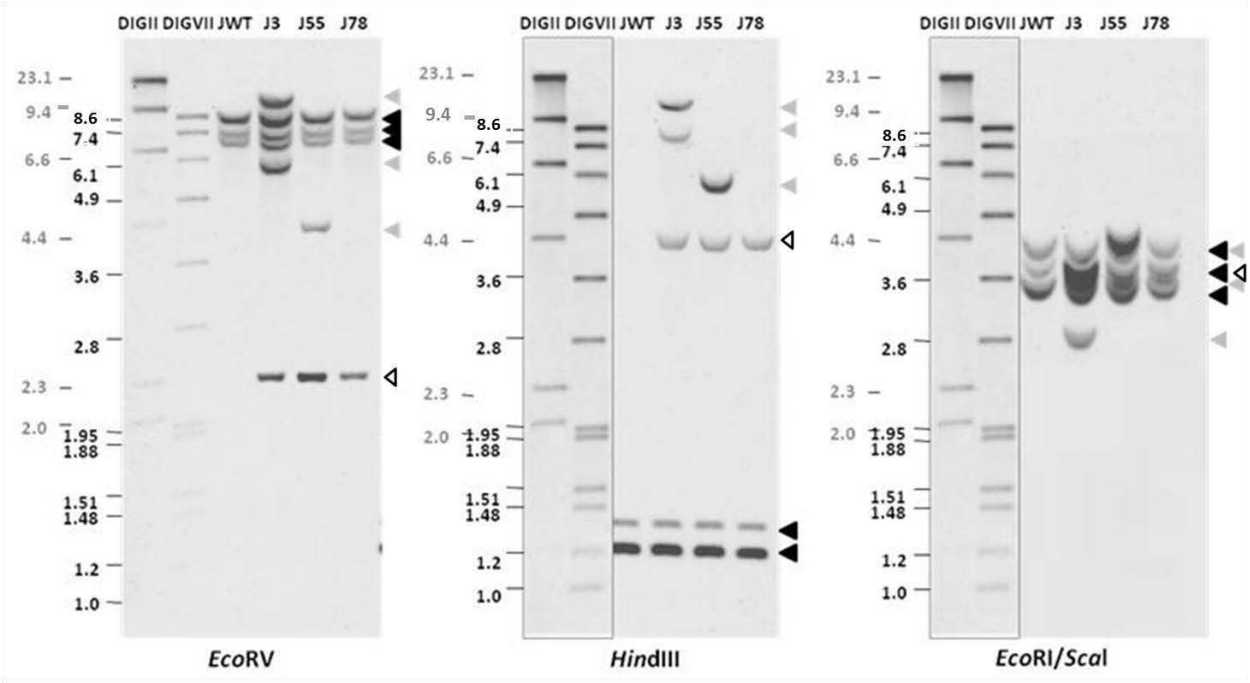
Genomic DNA of Atlantic control (JC) and events J3, J55 and J78 were digested and hybridized with the ASN probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 22. Atlantic Hybridization with the PHL Probe



Genomic DNA of Atlantic control (JC) and events J3, J55 and J78 were digested and hybridized with the PHL probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 23. Atlantic Hybridization with the GBS Probe



Genomic DNA of Atlantic control (JC) and events J3, J55 and J78 were digested and hybridized with the GBS probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Table 8.Atlantic Event J3: DNA Fragments Hybridizing with DNA Insert -Derived Probes

(Summary of observations from **Figures 20-23**)

Probe	Restriction Enzyme	Type of Band (kb)					Interpretation
		OB	**IB**	**IB**	**JB**	**JB**	
			Exp.	**Obs.**	**Exp.**	**Obs.**	
AGP Figure 20	EcoRV	8.0 5.2 2.1 1.4	2.3	2.3	1.1+	6.6 1.6*	**1.** ***Copy number at Left Border side*** Visualization of two **JBs** in all DNA digests with the AGP probe indicates that J3 contains two DNA insert copies (at least at the Left Border side). With the ASN probe, 3 **IBs (2.3, 0.7 - double intensity)** and 1 unexpected **JB (6.6)** were visualized in the *Eco*RV digestion. With the ASN probe in the *Hin*dIII digestion, we visualized 1 **IB (4.2)** and 1 unexpected **JB (7.8)** containing the ASN inverted repeat, which indicates the presence of two DNA insert copies at the LB side. With the ASN probe and EcoRV, the presence of a junction band indicates there is more than 1 copy.
	HindIII	16.0 8.5 7.0 6.0 2.2	4.2	4.2	2.4+	7.8 4.2*	
	EcoRI/ScaI	3.9 2.9 2.3 1.1	3.8	3.7	3.7+	6.6* 2.9*	**2.** ***Copy number at Right Border side*** Visualization of a single **JB** in all DNA digestions with the PHL probe indicates that J3 contains one DNA insert at the Right Border side. The two **JBs** in all DNA digests probed with GBS (together with one **IB**) indicates that J3 contains three pGbss elements, 2 in the single copy and 1 in the partial copy.
ASN Figure 21	EcoRV	1.4	2.3 0.7	2.3 0.7*[#]	none	6.6	
	HindIII	3.1	4.2	4.2	none	7.8	***3. Internal DNA insert Integrity*** Only one **IB** (no double intensity) was found when the three digestions (EcoRV, HindIII, EcoRI/ScaI) were probed with AGP and AGP2 (Figure 38, 2.3-kb), indicating that the second copy of DNA insert has a truncation in the internal AGP region. The deletion of the internal AGP includes both *Eco*RV sites (at 5,165 and 6,390). Additional evidence suggests a deletion of the internal AGP based on band intensity which indicates only three rather than four 1.2-kb bands in an *Eco*RV digest hybridized with the AGP3 probe (Figure 39). Instead of an expected second 2.3-kb **IB** in EcoRV digests with the ASN probe, we visualized a 6.6-kb fragment, indicating the deletion of *Eco*RV in one internal AGP promoter. Only one IB was shown in HindIII and EcoRI/ScaI digests hybridized with PHL, which indicates the presence of a single copy of DNA insert. Thus, the second copy lacks the PHL region. Visualization of three bands (1-IB, 2- JBs) hybridizing with the GBS probe indicates J3 contains three pGbss elements, 2 in the single copy and 1 in the partial copy.
	EcoRI/ScaI	8.4 7.4	none	none	3.7+	6.6*	
PHL Figure 22	EcoRV	18.0 12.0 7.4	none	none	3.6+	12.0	
	HindIII	18.0 8.6 6.0	1.3	1.3	2.2+	12.0	
	EcoRI/ScaI	1.6 1.5 1.3 1.1 0.9	3.8	3.8	1.9+	3.8	**4.** ***DNA insert Integrity at the extremities*** Both a 1.6-kb *Eco*RV band and a 4.2-kb *Hin*dIII band visualized with AGP are double intense, suggesting that two copies of the DNA insert are connected reversely at the LB side. In this case, a 2.2-kb EcoRV band including LB and partial AGP promoter from both DNA insert copies will be expected. However, only a 1.6-kb band was observed which means a 0.6- kb deletion from one or both DNA inserts occurred during the recombination. The partial DNA insert had a deletion at the RB side that extended beyond the first *Eco*RV site (5,165-bp) of the internal AGP promoter. One **JB** band in the EcoRI/ScaI digest that is visualized with the ASN probe confirmed that the two DNA inserts connected at the LB side. When digested with EcoRI/ScaI, we expected double intensity bands of AGP and ASN, with the same size of 6.6-kb, and confirmed the connection at the LB.

Table 8 (Continued). Atlantic Event J3: DNA Fragments Hybridizing with DNA Insert -Derived Probes
(Summary of observations from **Figures 20-23**)

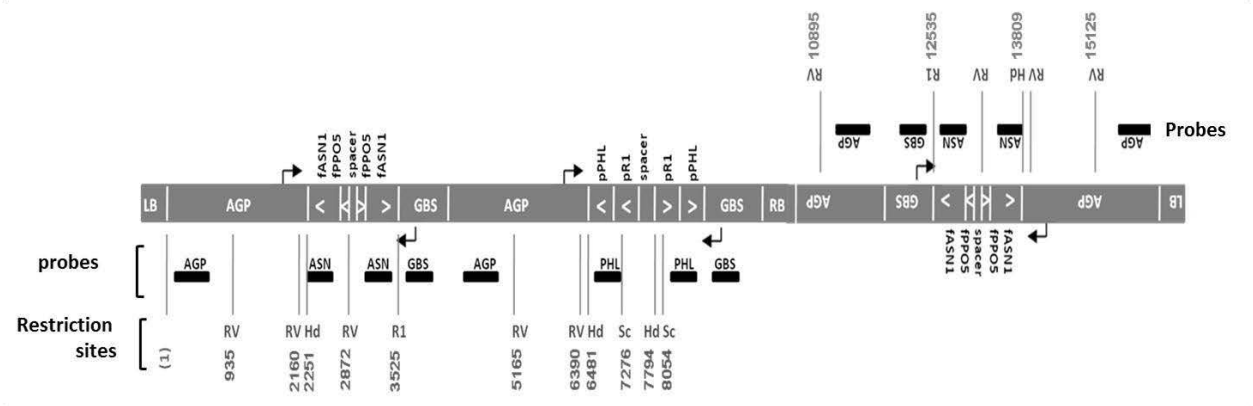
Probe	Restriction Enzyme	Type of Band (kb)					Interpretation
		OB	**IB**	**IB**	**JB**	**JB**	
			Exp.	**Obs.**	**Exp.**	**Obs.**	
GBS Figure 23	EcoRV	9.2 7.6 7.0	2.3	2.3	3.6+	12.0 6.1	***5. Variation between expected and observed sizes in DNA insert.*** Slight differences were found between the observed and expected size of a 3.8-kb internal band between restriction sites for EcoR1 at 3,525-bp and Sca1 at 7,276-bp. This band was expected with the AGP, PHL,and GBS probes with EcoR1 and Sca1, but appeared at 3.7-kb with AGP. This may be because of differences in gel loading or possibly the influence of plant material resulted in faster movement within the gel. See further discussion in section titled "Additional Studies on DNA insert Integrity for all Events."
	HindIII	1.3 1.2	4.2	4.2	2.2+	12.0 8.4	
	EcoRI/ScaI	4.3 3.8 3.5	3.8	3.8	1.9+	3.8 2.9	

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with "*" were double intensity. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Copy Number and Integrity: Atlantic Event J55

J55 contains one full copy of the DNA insert, and an additional truncated and linked copy that lacks the R1/PHL promoter silencing cassette (see **Figure 24**). This conclusion was deduced from hybridizations shown in **Figures 20-23** which are summarized in **Table 9**.

Figure 24. J55 Insert



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII

Two observed **JBs** at the Left Border side in the EcoRV digest hybridized with the AGP probe represented two copies of the DNA insert. Indeed, the presence of 2 copies at the Left Border side is confirmed by the *Eco*RI/*Sca*I digest hybridized with the ASN probe. However, only one **JB** is visualized at the Right Border side, indicating that only one copy of the DNA insert has the Right Border region. A truncation occurred within the central AGP promoter which removed all sequences at the right of the central AGP promoter. Also, about 400-bp of the central AGP promoter itself (between the *Eco*RV and *Hin*dIII sites) is missing because the 2.3-kb **IB** visualized when hybridizing an *Eco*RV digest with the AGP probe is still present. Confirmation for the absence of sequences to the right of the central AGP promoter comes from the fact that the numbers of **IB** and **JB** visualized with the PHL probe are just enough for one intact DNA insert copy, indicating that the PHL region in the second copy of DNA insert is missing. Since only one **JB** and two **IB** identified in an EcoRV digest with GBS probe, the Gbss promoter at the Right Border side is not present in one of the DNA inserts.

Furthermore, a 6-kb *Hin*dIII **JB** was shown in all the hybridizations with the AGP, ASN, PHL and GBS probes, indicating that the RB border of the complete DNA insert is reversely connected to the central AGP promoter of the truncated DNA insert. This structure (see **Figure 20**) is supported by the presence of 4.5-kb *Sca*I-*EcoRI* AGP band and a 4.5-kb *Sca*I-*EcoRI* GBS band that was double intense because of two copies of GBS promoters. This connection of two DNA inserts is also supported by the fact that a 4.5-kb band connecting 2 DNA inserts was observed when an *Eco*RV digest was hybridized with AGP3 probe (**Figure 39**). The junction between the two DNA inserts was further confirmed by sequence analyses described in **Appendix 4. Junction Analysis and Event-Specific Detection**.

Table 9. Atlantic Event J55: DNA Fragments Hybridizing with DNA Insert-Derived Probes
(Summary of observations from **Figures 20-23**)

Probe	Restriction Enzyme	Type of Band (kb)				
		OB	**IB**	**IB**	**JB**	**JB**
			Exp.	**Obs.**	**Exp.**	**Obs.**
AGP Figure 20	EcoRV	8.0 5.2 2.1 1.4	2.3	2.3*	1.1+	5.3 2.0
	HindIII	16.0 8.5 7.0 6.0 2.2	4.2	4.2	2.4+	6.7 5.8 3.4
	EcoRI/ScaI	3.9 2.9 2.3 1.1	3.8	3.7	3.7+	5.0 4.5 3.8
ASN Figure 21	EcoRV	1.4	2.3 0.7	2.3* 0.7#*	none	none
	HindIII	3.1	4.2	4.2	None	5.8
	EcoRI/ScaI	8.4 7.4	None	none	3.7+	5.3 4.0
PHL Figure 22	EcoRV	18.0 12.0 7.4	None	none	3.6+	4.4
	HindIII	18.0 8.6 6.0	1.3	1.3	2.2+	5.7
	EcoRI/ScaI	1.6 1.5 1.3 1.1 0.9	3.8	3.8	1.9+	4.8
GBS Figure 23	EcoRV	9.2 7.6 7.0	2.3	2.3*	3.6+	4.4
	HindIII	1.3 1.2	4.2	4.2	2.2+	6.1*
	EcoRI/Sca	4.3 3.8 3.5	3.8	3.8	1.9+	4.5*

Interpretation

1. ***Copy number at Left Border side***
Visualization of two **JB** in *Eco*RV digest with AGP probe and *Eco*RI/*Sca*I digest with ASN probe indicates that J55 contains two DNA inserts (at least at the Left Border side).

2. ***Copy number at Right Border side***
Visualization of a single **JB** in with the PHL and GBS probes indicates that J55 contains one DNA insert at the Right Border side.

3. ***Internal DNA insert Integrity***
There are four bands (**IB** and **JB**) found in all three digests probed with either AGP or ASN, which means that J55 contains two copies of the DNA insert. However, only one **IB** (4.2) hybridizing with AGP was observed in the *Hin*dIII digestion, indicating that the associated AGP promoter was truncated. The truncated region is about 400-bp with a breakpoint between *Eco*RV (6,390) and *Hin*dIII (6,481) sites in the partial copy because the 2.3-kb *Eco*RV **IB** hybridizing with AGP is still present. The number of bands visualized in all three digests probed with PHL indicates the presence of one intact copy of the DNA insert (thus, the second copy lacks PHL-hybridizing sequences). Visualization of three bands in all digests hybridizing with the GBS probe indicates J55 contains three pGbss elements, 2 in the single copy and 1 in the partial copy.

4. ***DNA insert Integrity at the extremities***
In the HindIII digests, a band was shown that ranged from 5.7 to 6.1 in the hybridizations probed with AGP, ASN, PHL and GBS. These are all the same band but show slight variations with the different probes. This suggests the association of two DNA insert copies, whereby the RB border of the first copy is linked to a truncated pAGP of the second copy.

The second DNA insert is in the reverse orientation. Evidence for this structure comes from the presence of (1) a 4.5-kb *Sca*I-*Eco*RI band hybridizing with AGP, (2) a double-intense 4.5-kb *Sca*I/*Eco*RI GBS-hybridizing band, and (3) a double-intense 6.0-kb HindIII GBS band. Furthermore, a 4.5-kb band is visualized when *Eco*RV digest is hybridized with the AGP3 probe (Figure 39).

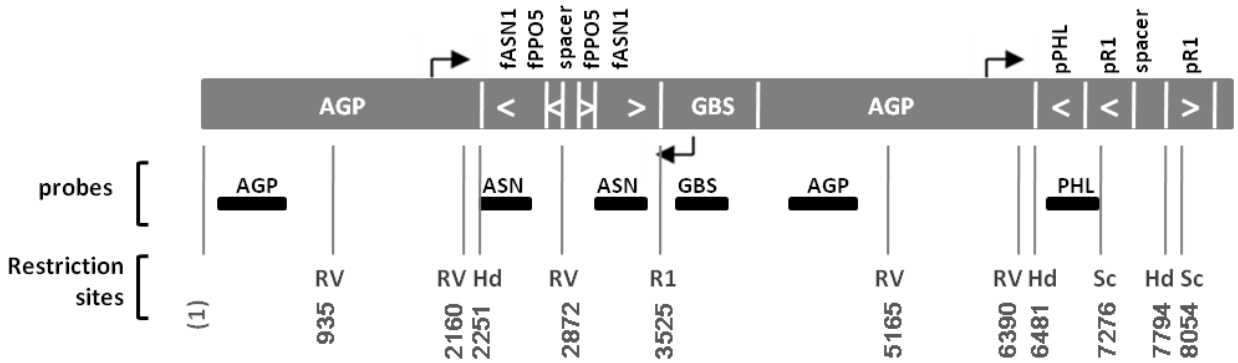
5. ***Variation between expected and observed sizes in DNA insert.*** Slight differences were found between the observed and expected size of a 3.8-kb internal band between restriction sites for EcoR1 at 3,525-bp and Sca1 at 7,276-bp. This band was expected with the AGP, PHL,and GBS probes with EcoR1 and Sca1, but appeared at 3.7-kb with AGP. This may be because of differences in gel loading or possibly the influence of plant material resulted in faster movement within the gel. See further discussion in section titled "Additional Studies on DNA insert Integrity for all Events."

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with "*" were double intensity. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Copy Number and Integrity: Atlantic Event J78

J78 contains a single truncated DNA insert (see **Figure 25**). This conclusion was deduced from hybridizations shown in **Figures 16-19** which are summarized in **Table 10**.

Figure 25. J78 Insert



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII

Visualization of a single **JB** when *Eco*RI, *Hin*dII and *Eco*RI/*Sca*l digests are hybridized with the AGP probe demonstrates that J78 contains a single copy of the DNA insert at the Left Border side. However, there is a truncation at the Left Border side because the **JB** is 200-bp shorter than the predicted minimum size when *Eco*RI/*Sca*l digests are hybridized with AGP or ASN probe. Using GBS as a probe, only one **IB** was visualized while the expected **JB** band on Right Border side was not found in all three digestions, indicating that the Gbss promoter adjacent to the Right Border was deleted. PHL hybridization further proved that the whole or partial PHL fragment next to Gbss promoter was also missing in both *Hin*dIII and *Eco*RI/*Sca*l digestions. Indeed, the junction sequence analysis demonstrated that 486-bp of PHL was deleted as described in **Appendix 4. Junction Analysis and Event-Specific Detection**.

Table 10. Atlantic Event J78: DNA Fragments Hybridizing with DNA Insert-Derived Probes
(Summary of observations from **Figures 20-23**)

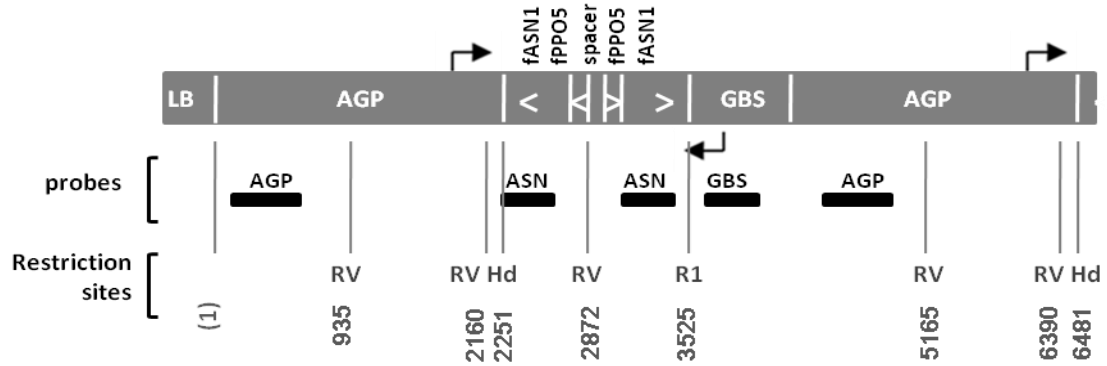
Probe	Restriction Enzyme	Type of Band (kb)					Interpretation
		OB	**IB Exp.**	**IB Obs.**	**JB Exp.**	**JB Obs.**	
AGP Figure 20	EcoRV	8.0 5.2 2.1 1.4	2.3	2.3	1.1+	12.0	**1.** ***Copy number at Left Border side*** Visualization of a single **JB** in the all digestions with AGP probe and EcoR/ScaI with ASN probe indicates that J78 contains one copy of DNA insert (at least the Left Border side).
	HindIII	16.0 8.5 7.0 6.0 2.2	4.2	4.2	2.4+	2.1	**2.** ***Copy number at Right Border side*** J78 contains a single DNA insert without the Right Border because only internal PHL and GBS bands were visualized.
	EcoRI/ScaI	3.9 2.9 2.3 1.1	3.8	3.7	3.7+	3.5	**3.** ***Internal DNA insert Integrity*** Integrity of the DNA insert is indicated by the fact that the **IBs** visualized in various DNA digests have nearly the expected sizes (see note 5 below regarding variation). See also Figure 40 for 0.7-kb IB.
ASN Figure 21	EcoRV	1.4	2.3 0.7	2.3 0.7#	None	none	**4.** ***DNA insert Integrity at the extremities*** **JB** bands are about 200-bp shorter than the expected sizes when genomic DNA was digested with either *Hin*dIII or *Eco*R1/*Sca*I and probed with either AGP or ASN, indicating that there is a truncation at the Left Border region. In addition, J78 suffers a deletion of about 486-bp comprising PHL and GBS-hybridizing sequences on the right border side.
	HindIII	3.1	4.2	4.2	None	none	
	EcoRI/ScaI	8.4 7.4	None	none	3.7+	3.5	
PHL Figure 22	EcoRV	18.0 12.0 7.4	None	none	3.6+	3.0	
	HindIII	18.0 8.6 6.0	1.3	1.3	2.2+	none	**5.** ***Variation between expected and observed sizes in DNA insert.*** Slight differences were found between the observed and expected size of a 3.8-kb internal band between restriction sites for EcoR1 at 3,525-bp and Sca1 at 7,276-bp. This band was expected with the AGP, PHL,and GBS probes with EcoR1 and Sca1, but appeared at 3.7-kb with AGP. This may be because of differences in gel loading or possibly the influence of plant material resulted in faster movement within the gel. See further discussion in section titled "Additional Studies on DNA insert Integrity for all Events."
	EcoRI/ScaI	1.6 1.5 1.3 1.1 0.9	3.8	3.8	1.9+	none	
GBS Figure 23	EcoRV	9.2 7.6 7.0	2.3	2.3	3.6+	none	
	HindIII	1.3 1.2	4.2	4.2	2.2+	none	
	EcoRI/ScaI	4.3 3.8 3.5	3.8	3.8	1.9+	none	

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Copy Number and Integrity: G Event G11

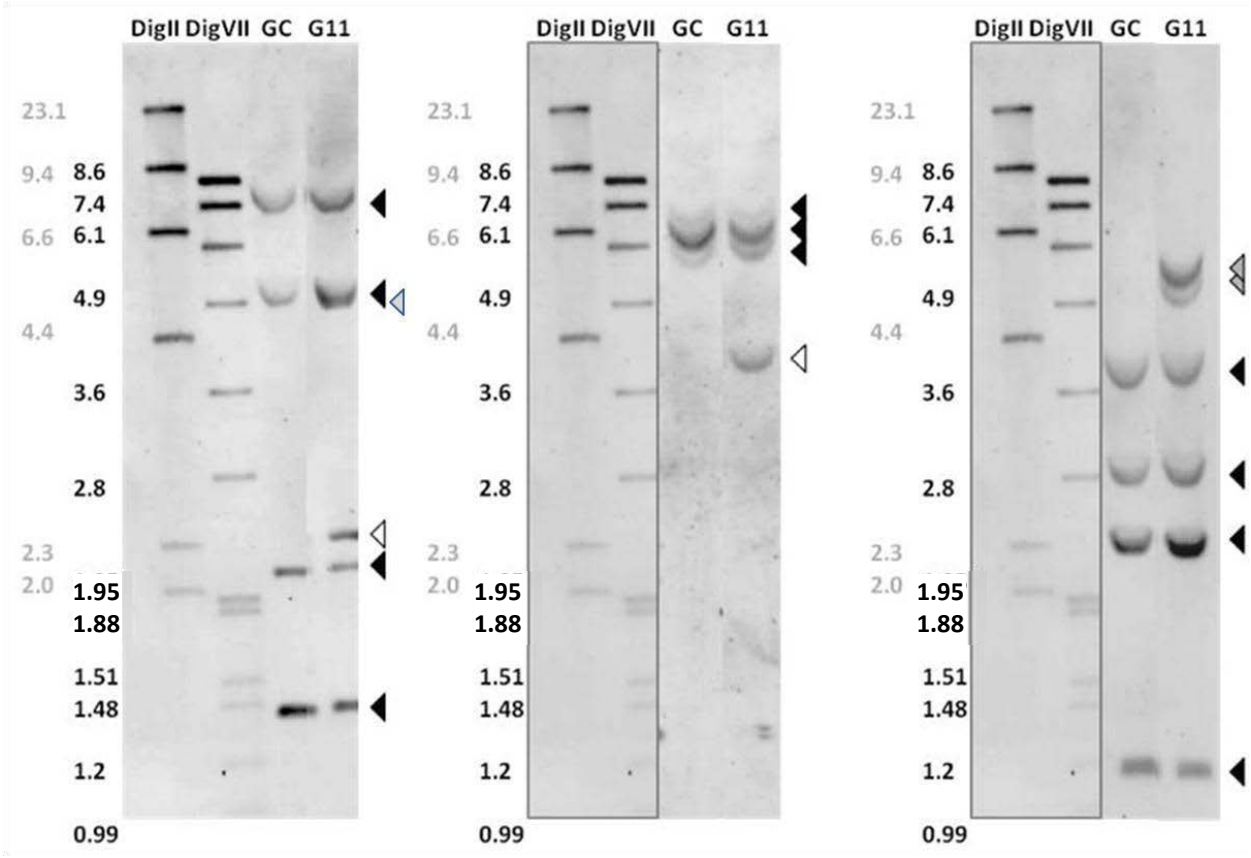
The hybridization data for event G11 are shown in **Figures 27-30** and summarized in **Table 11**. In summary, a single **JB** in AGP and ASN hybridizations indicated that G11 contains a single DNA insert with an intact Left Border (See **Figure 26**). No **JB** was observed in DNA digests hybridized with the PHL probe and *Eco*RV and *Hin*dIII digests hybridized with the GBS probe. This indicated that G11 does not contain an insert with a Right Border. Observation of **IBs** with sizes that are different from the predicted sizes for *Eco*RI/*Sca*I digests when hybridized with AGP, PHL and GBS probes confirmed that the Right Border side of the DNA insert was missing. The truncation occurred on the Right Border side, upstream from the *Sca*I site at position 7,276-bp. A junction study described in **Appendix 4. Junction Analysis and Event-Specific Detection** showed that the actual truncation site was at position 6,618-bp within the DNA insert. A summary of the deduced insert for G11 is shown below. Event G11 does not have the pPHL-pR1 inverted repeat DNA. The second AGP promoter is immediately upstream from the DNA insert/plant DNA junction.

Figure 26. G11 Insert



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII

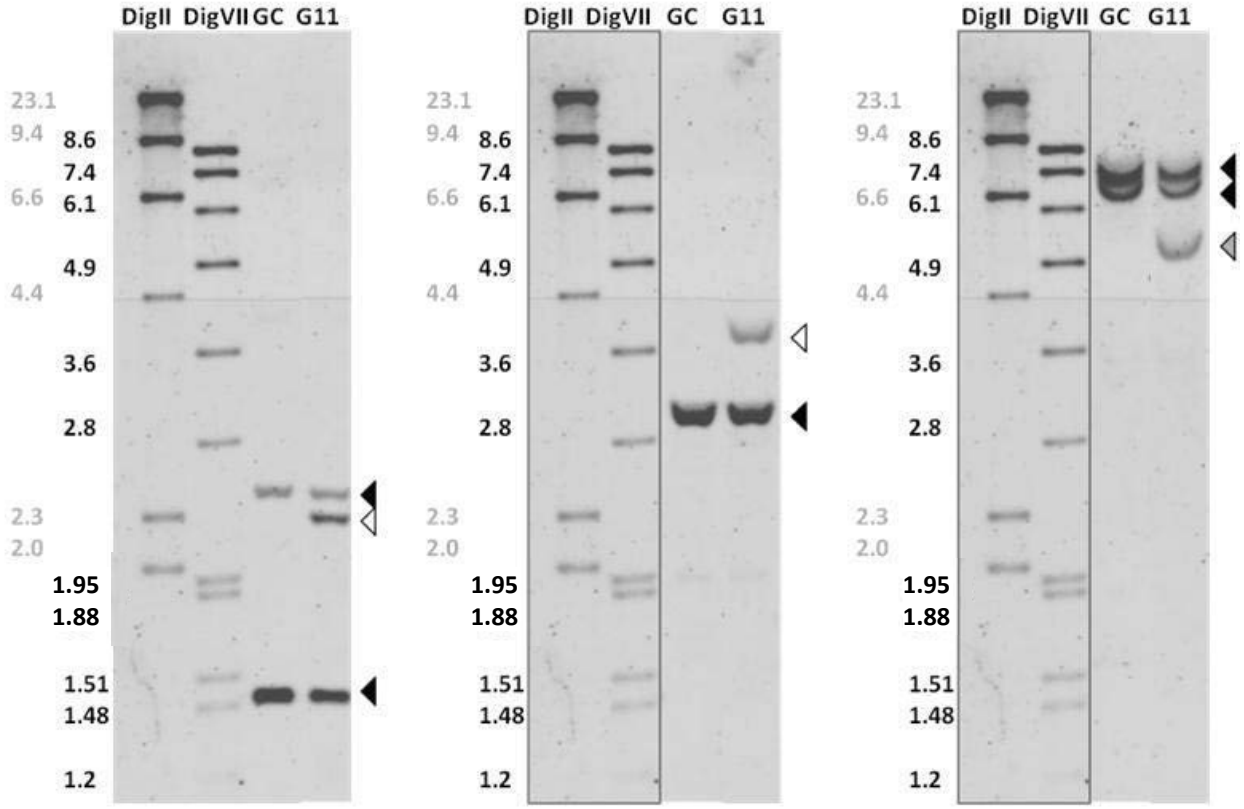
Figure 27. G Event DNA Hybridization with the AGP Probe



*Eco*RV *Hin*dIII *Eco*RI/*Sac*I

Genomic DNA of G control (GC) and event G11 was digested and hybridized with the AGP probe. The restriction enzymes used are indicated below each panel. Size of the DigII and DigVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb) in gray and black, respectively. Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

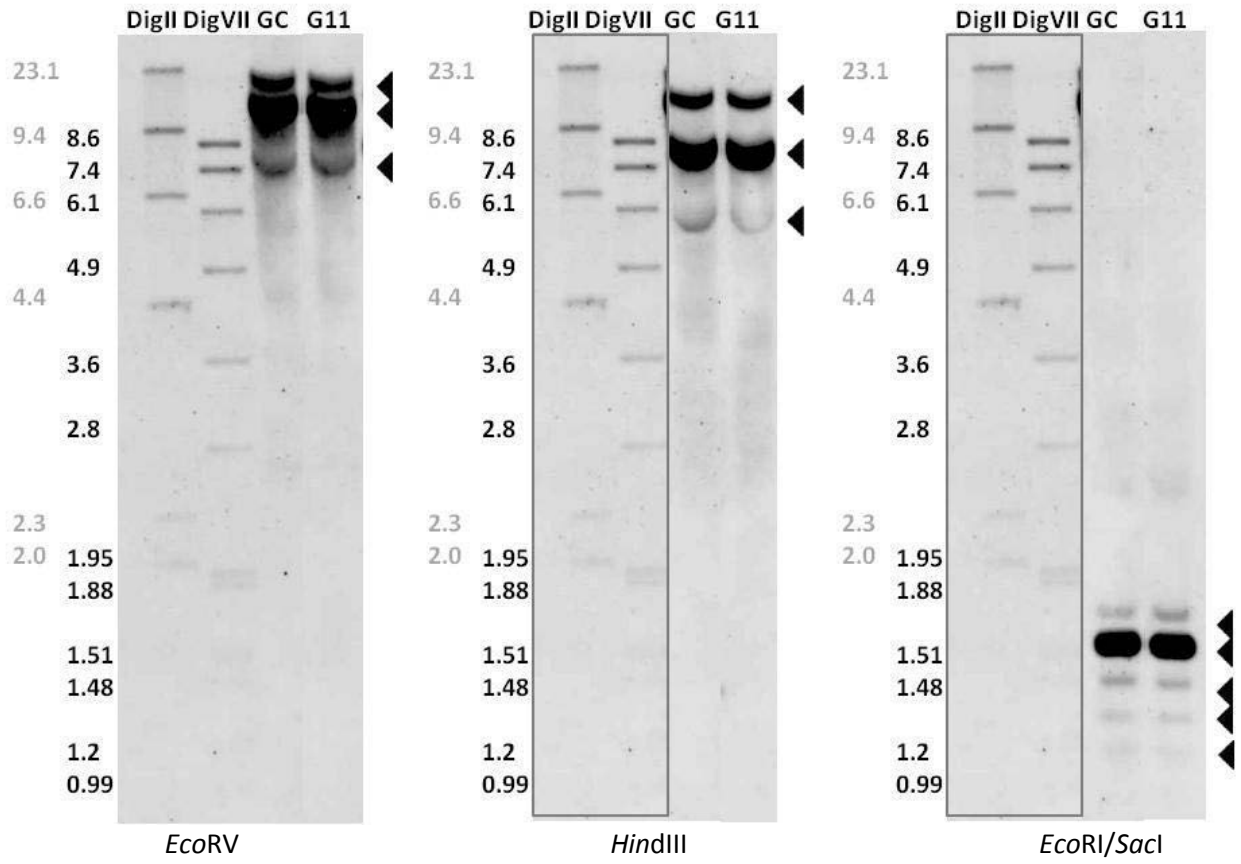
Figure 28. G DNA Hybridization with the ASN Probe



*Eco*RV *Hin*dIII *Eco*RI/*Sac*I

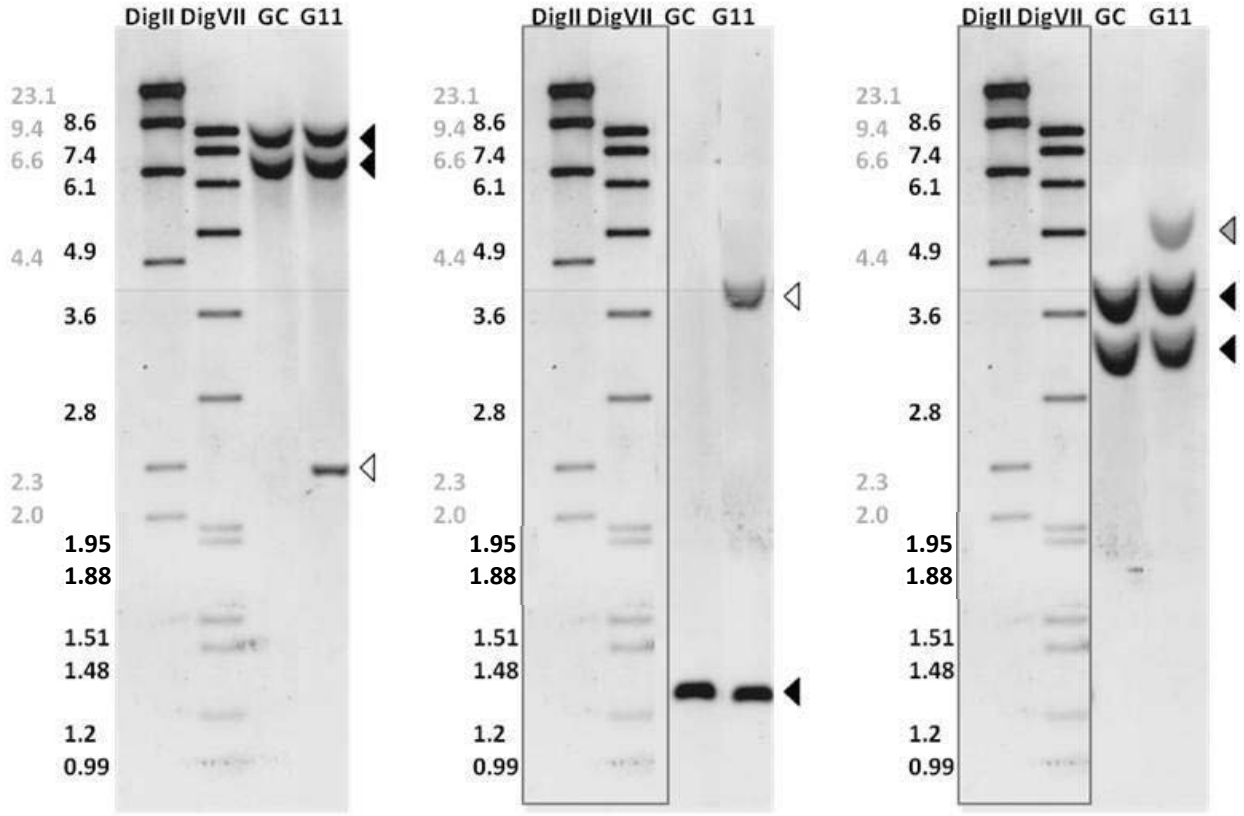
Genomic DNA of G control (GC) and event G11 was digested and hybridized with the ASN probe. The enzymes used are indicated below each panel. Size of the DigII and DigVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb) in gray and black, respectively. Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 29. G DNA Hybridization with the PHL Probe



Genomic DNA of G control (GC) and event G11 was digested and hybridized with the PHL probe. The enzymes used are indicated below each panel. Size of the DigII and DigVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb) in gray and black, respectively. Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**. Observations are summarized in **Table 8**.

Figure 30. G DNA hybridization with the GBS probe



*Eco*RV *Hin*dIII *Eco*RI/*Sac*I

Genomic DNA of G control (GC) and event G11 was digested and hybridized with the GBS probe. The enzymes used are indicated below each panel. Size of the DigII and DigVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb) in gray and black, respectively. Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Table 11. G Event G11: DNA Fragments Hybridizing with DNA Insert-Derived Probes
(Summary of observations from **Figures 27-30**)

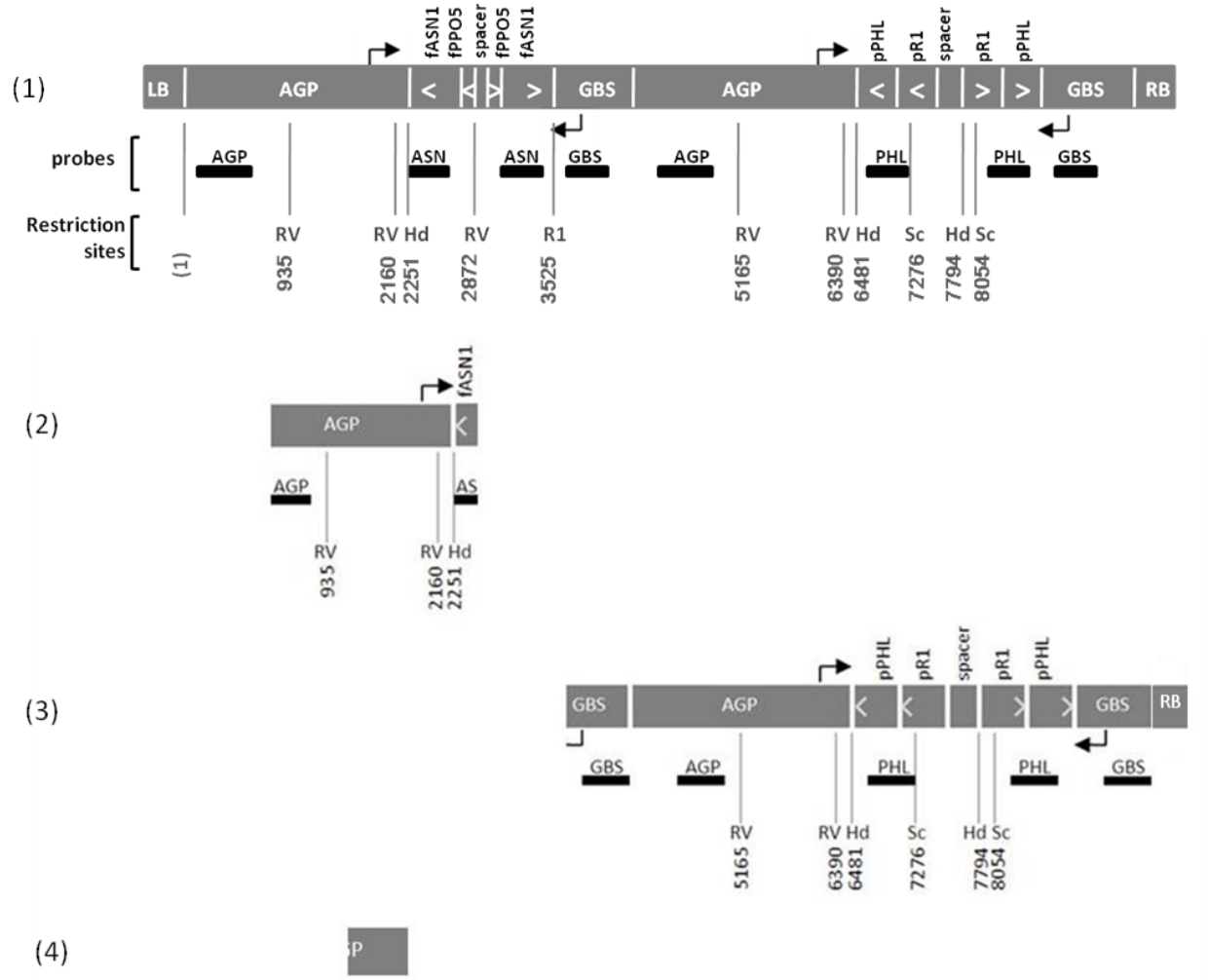
Probe	Restriction Enzyme	Type of Band (kb)					Interpretation
		OB	**IB**	**IB**	**JB**	**JB**	
			Exp.	**Obs.**	**Exp.**	**Obs.**	
AGP Figure 27	EcoRV	8.4 5.3 2.1 1.5	2.3	2.3	1.1+	5.3*	**1.** ***Copy number at Left Border side*** Visualization of a single **JB** in each of EcoRV digested AGP hybridizations and EcoRI/ScaI digested ASN hybridization indicates that G11 contains a single DNA insert (containing the Left Border side). Although 2 bands were observed in EcoRI/ScaI digested AGP hybridization, the absence of an IB in this digest indicates one of the bands is the truncated IB. *The double intensity band at about 5.3-kb appears to include both the 5.3-kb OB and the 5.3-kb JB **2.** ***Copy number at Right Border side*** No **JB** was **o**bserved in each DNA digest with PHL hybridization and EcoRV, HindIII, or EcoRI/ScaI. Digests with GBS hybridization indicated that G11 does not contain the Right Border DNA insert. The missing IB from the EcoRI/ScaI digest with GBS hybridization and the visible 5.3-kb JB is indicative of the truncated IB and confirms there is only one copy of the Gbss fragment in the DNA insert at the Left Border side of the construct. **3.** ***Internal DNA insert Integrity*** **IBs** with expected sizes observed in EcoRV or HindIII digests with AGP and GBS hybridizations indicated the integrity of the DNA insert to the second HindIII site (6,481-bp position on pSIM1278). The absence of IBs for the EcoRI/ScaI digest with AGP and GBS hybridizations indicated that the Right Border side of the DNA insert was truncated at a point before the first *Sca*I site (upstream from 7,276-bp). **4.** ***DNA insert Integrity at the extremities*** No **IB** was visualized in the digest hybridized with the GBS probe and EcoRI/ScaI, indicating that the DNA insert for G11 was truncated between the HindIII site at 6,481-bp and the ScaI site at 7,276-bp. No **JB** was observed when PHL probes for the right border side of the DNA insert were used with *all three* digests. This indicated that a portion of the DNA insert was missing from the right border side of the DNA insert, including the PHL element. The absence of the IB in the EcoRI/ScaI digests probed with PHL suggests that no intact PHL sequences are present in the truncated insert.
	HindIII	7.0 6.7 6.1	4.2	4.2	2.4+	None	
	EcoRI/ScaI	4.2 3.0 2.5 1.2	3.8	none	3.7+	5.9 5.5	
ASN Figure 28	EcoRV	2.5 1.5	2.3 0.7	2.3 0.7#	None	None	
	HindIII	3.1	4.2	4.2	None	None	
	EcoRI/ScaI	7.6 7.0	none	none	3.7+	5.5	
PHL Figure 29	EcoRV	20.0 15.0 8.0	none	none	2.2+	none	
	HindIII	15.0 8.6 5.8	1.3	none	3.6+	none	
	EcoRI/ScaI	1.7 1.6 1.5 1.3 1.2	3.8	none	1.9+	none	
GBS Figure 30	EcoRV	8.6 7.0	2.3	2.3	3.6+	none	
	HindIII	1.3	4.2	4.2	2.2+	none	
	EcoRI/ScaI	4.2 3.4	3.8	none	1.9+	5.3	

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with "*" were double intensity. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Copy Number and Integrity: H Event H37

The hybridization data for event H37 are shown in **Figures 32-35** and summarized in **Table 12**. Event H37 contains one complete DNA insert, two truncated inserts, and one additional small fragment only comprising a part of the AGP promoter (see **Figure 31** below). To simplify explanations, these copies have been labeled as copy number 1, 2, 3, and 4 respectively.

Figure 31. H37 Inserts



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII
Diagram (4) is a small fragment containing part of the AGP promoter.

At least one copy of the DNA insert is complete as indicated by the **IBs** and **JBs** in all the DNA digests that have the expected sizes. Two extra JBs (one was expected, 3 total) were observed when EcoRV digests were hybridized with the AGP probe, indicating two more DNA insert copies are in H37. One of the copies is truncated at the first Asn1 fragment downstream from the Left Border. This is indicated by only

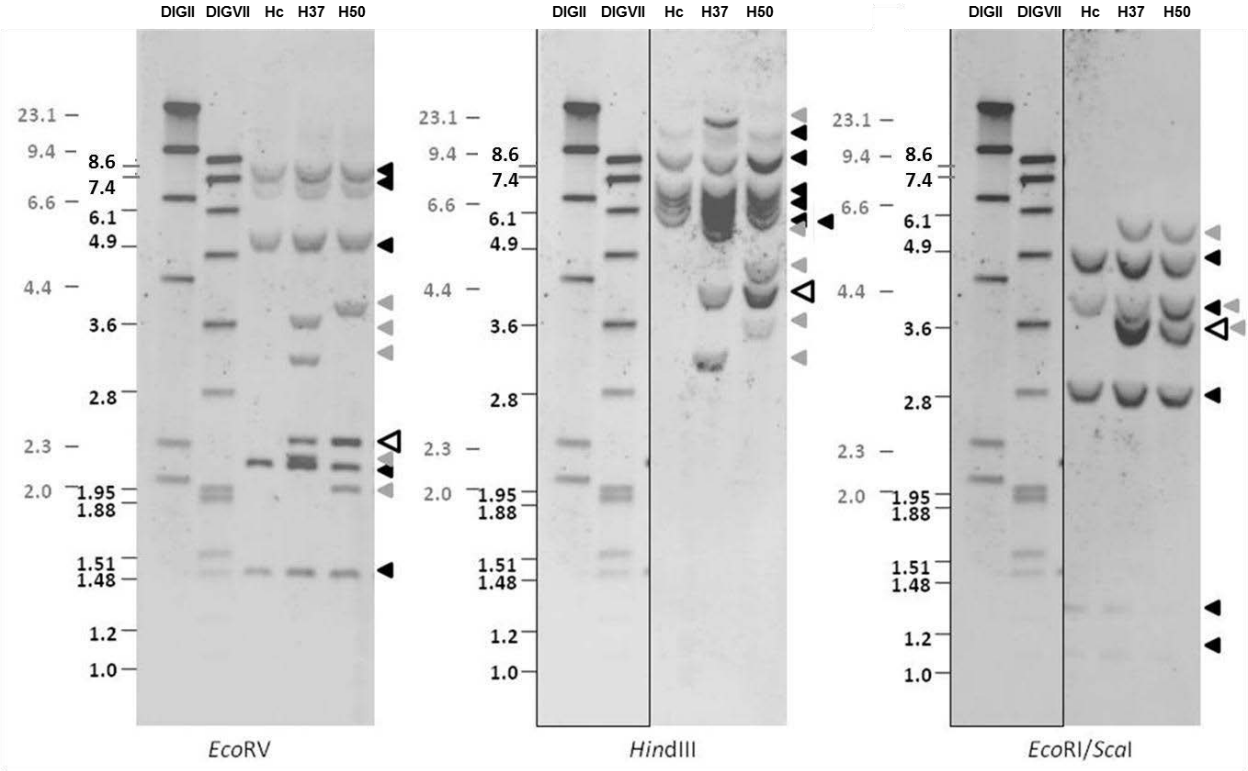
one copy of **IBs (**0.7-kb, 2.3-kb) visualized when the EcoRV digests were hybridized with the ASN probe (**Figures 33 and 39**) and the observation of **JBs** with *Eco*RV and *Hin*dIII when probed with ASN.

H37 possesses a third partial DNA insert containing the Right Border side. This is indicated by the two **JBs** and two **IBs** observed in the *Hin*dIII digest with the PHL probe. Only one **IB** (3.7-kb) was observed when *Eco*R1/*Sca*l digests were hybridized with GBS and AGP probes, which indicated that this third DNA insert was missing the Left Border region and started at the central GBS near the *Eco*R1 site, extending to the Right Border.

In addition, a very faint band with a size of about 4.2-kb, indicating the presence a small portion of part of an AGP promoter, was detected in H37 with the AGP3 probe (**Figure 34**). This fragment was not visualized with the AGP or AGP2 probe, indicating that the insert itself is smaller than 1.0-kb and has been depicted as copy number 4. A similar ~4.2-kb band was identified in event G9, which is not part of this submission.

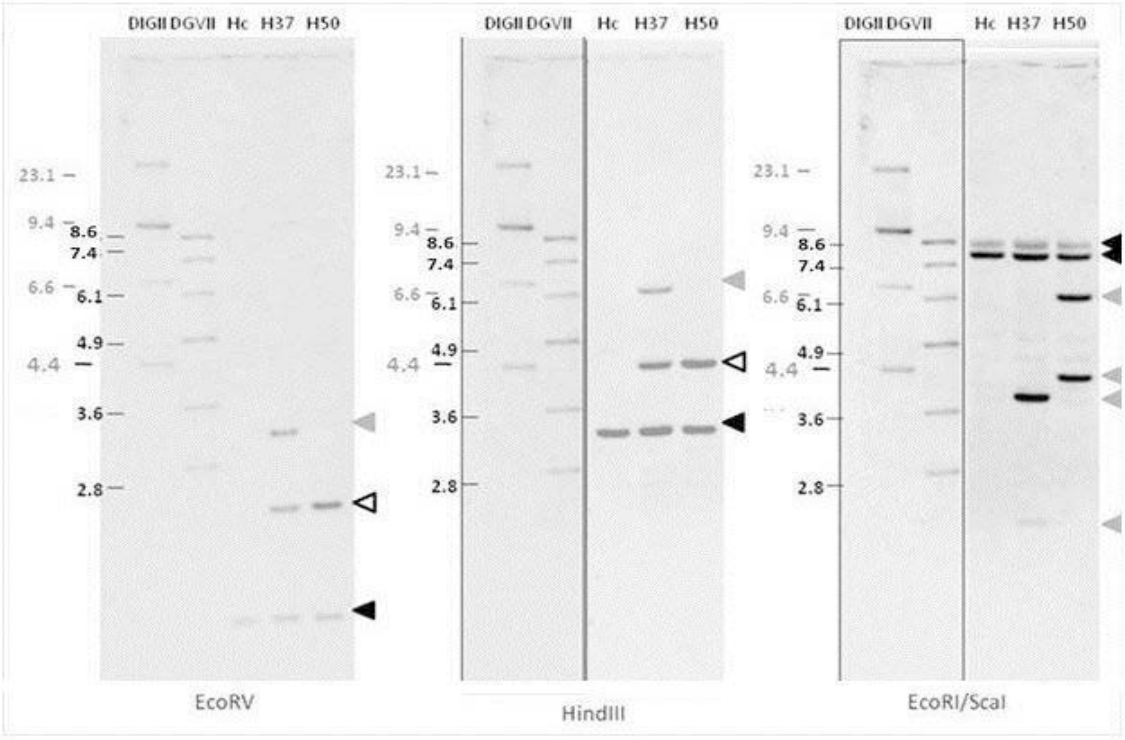
In summary, the deduced inserts for event H37 are shown above. This event is believed to have four inserts. One is a full-length DNA insert. The second is a shortened insert with only one AGP promoter and a portion of one ASN1 fragment. The third insert is also a shortened DNA insert containing only the second inverted repeat with the pPHL and pR1 fragment inverted repeats and the Right Border. The fourth insert consists of a part of the AGP promoter only.

Figure 32. H Hybridization with the AGP Probe



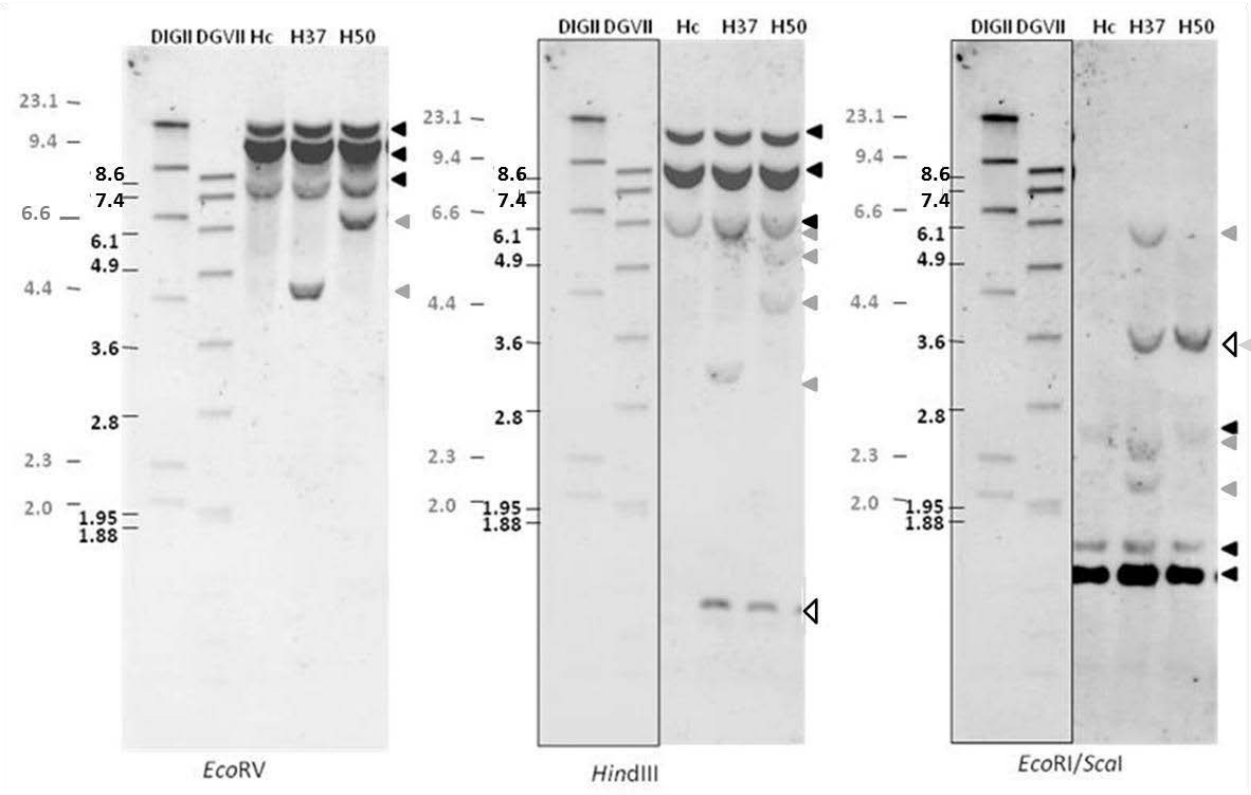
Genomic DNA of H control (Hc) and events H37 and H50 were digested and hybridized with the AGP probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb) in gray and black, respectively. Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 33. H Hybridization with the ASN Probe



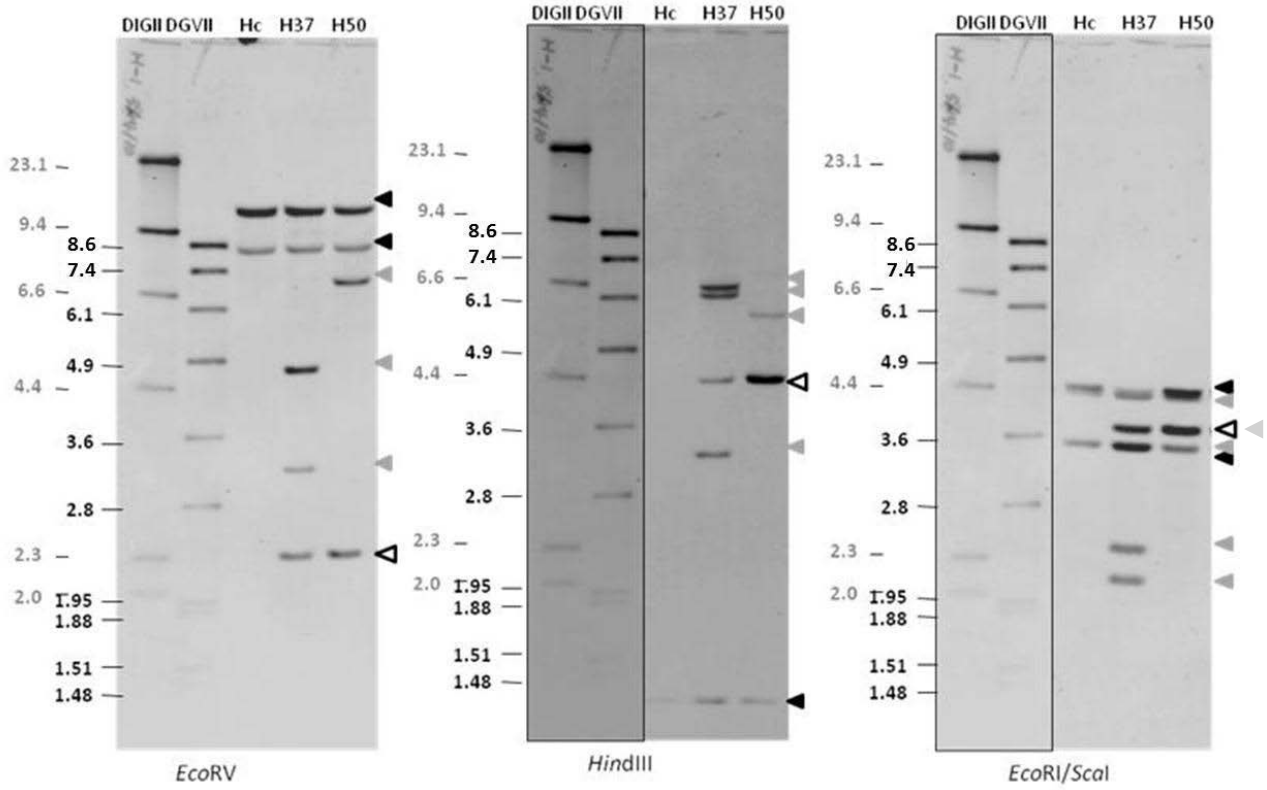
Genomic DNA of H control (Hc) and events H37 and H50 were digested and hybridized with the ASN probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb) in gray and black, respectively. Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 34. H Hybridization with the PHL Probe



Genomic DNA of H control (Hc) and events H37 and H50 were digested and hybridized with the PHL probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb) in gray and black, respectively. Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Figure 35. H Hybridization with the GBS Probe



Genomic DNA of H control (Hc) and events H37 and H50 were digested and hybridized with the GBS probe. The enzymes used are indicated below each panel. Size of the DIGII and DIGVII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb) in gray and black, respectively. Lanes for all three enzymes were on one blot originally, and were separated into three segments (one for each digest) with the marker lanes copied and boxed for better illustration. The original bands (**OBs**) are indicated by "◀", the internal bands (**IBs**) are indicated by "◁", and the junction bands (**JBs**) are indicated by "◀". The definitions for **OBs**, **IBs** and **JBs** can be found in the **Introduction**.

Table 12. H Event H37: DNA Fragments Hybridizing with DNA Insert-Derived Probes
(Summary of observations from **Figures 32-35**)

Probe	Restriction Enzyme	Type of Band (kb)					Interpretation
		OB	**IB**	**IB**	**JB**	**JB**	
			Exp.	**Obs.**	**Exp.**	**Obs.**	
AGP Figure 32	EcoRV	8.0 7.2 5.4 2.2 1.5	2.3	2.3	1.1+	3.8 3.2 2.2	
	HindIII	12.0 8.6 7.0 6.6 6.1 5.8	4.2	4.2	2.4+	20 5.8 3.3	
	EcoRI/ScaI	4.9 4.3 3.0 1.3 1.1	3.8	3.7	3.7+	5.8 3.5*	
ASN Figure 33	EcoRV	1.4	2.3 0.7	2.3 0.7#	none	3.3	
	HindIII	3.4	4.2	4.2	none	6.4	
	EcoRI/ScaI	9.4 8.6	none	none	3.7+	3.7 2.4 (weak)	
PHL Figure 34	EcoRV	20.0 14.0 8.2	none	none	3.6+	4.7*	
	HindIII	16.0 9.2 6.5	1.3	1.3*	2.2+	6.5 3.3	
	EcoRI/ScaI	2.4 1.8 1.7	3.8	3.7	1.9+	5.8 2.4 2.1	
GBS Figure 35	EcoRV	12 8.4	2.3	2.3	3.6+	4.7* 3.2	
	HindIII	0.9	4.2	4.2	2.2+	6.5 6.1 3.3	
	EcoRI/ScaI	4.3 3.6	3.8	3.8	1.9+	3.5 2.4 2.1	

1. ***Copy number at Left Border side***
H37 showed three JB bands with the *Eco*RV and *Hin*dIII digests using the AGP probe. This indicated three copies of the insert. However, the *Eco*RI/*Sca*I digest with the AGP probe gave only two **JB** bands, which suggested that one of the three copies was truncated at the left border side.

2. ***Copy number at Right Border side***
H37 contains two copies of the DNA insert extending to the Right Border side as viewed with the PHL and GBS enzymes. However, it contains three rather than two JBs with *Eco*RV. The third JB (in bold, EcoRV with GBS) is smaller than the minimum size expected, and was generated by truncation of one of the two DNA inserts at a site within the centrally-located pGbss.

3. Internal DNA insert Integrity
The first DNA insert copy (DNA insert1) is intact because nearly all **IBs** visualized in various DNA digests have expected sizes (see comment 5 below). The second copy (DNA insert2) contains the first AGP promoter and is truncated within the Asn1-derived sequence, because the expected IBs for a second complete copy were not found. Copy number 3, DNA insert3, is truncated as it misses the Left Border side, and lacks additional IBs (2.3, 0.7) associated with the ASN fragments when digested with *Eco*RV and hybridized with the ASN probe.

4. DNA insert Integrity at the extremities
DNA insert1 is complete because the **JBs** are greater than the minimum size expected. Evidence for the structure of DNA insert2 were the JBs found with *Eco*RV and *Hin*dIII digests with the ASN probe when none are expected. DNA insert2 is truncated at Asn1 as indicated by visualization of a **JB** band that was unexpected and smaller in size (2.4) with the ASN probe in the *Eco*RI/ScaI digest. The total size of this copy should be smaller than 2.4-kb since *Eco*RI/*Sca*I would cut outside of the proposed DNA insert2. DNA insert 3 is missing the left side as indicated by a smaller than expected band (3.2-kb, in bold) in the *Eco*RV digestion with GBS probe. Also, DNA insert 3 has one **IB** band plus 3 **JB** bands in other digestions with *Eco*RI/*Sca*I, and both GBS and PHL probes. These 3-JBs that hybridize with the PHL and GBS probes are found instead of the expected IBs, indicating a truncation to the right of the EcoR1 site at 3,525-bp.

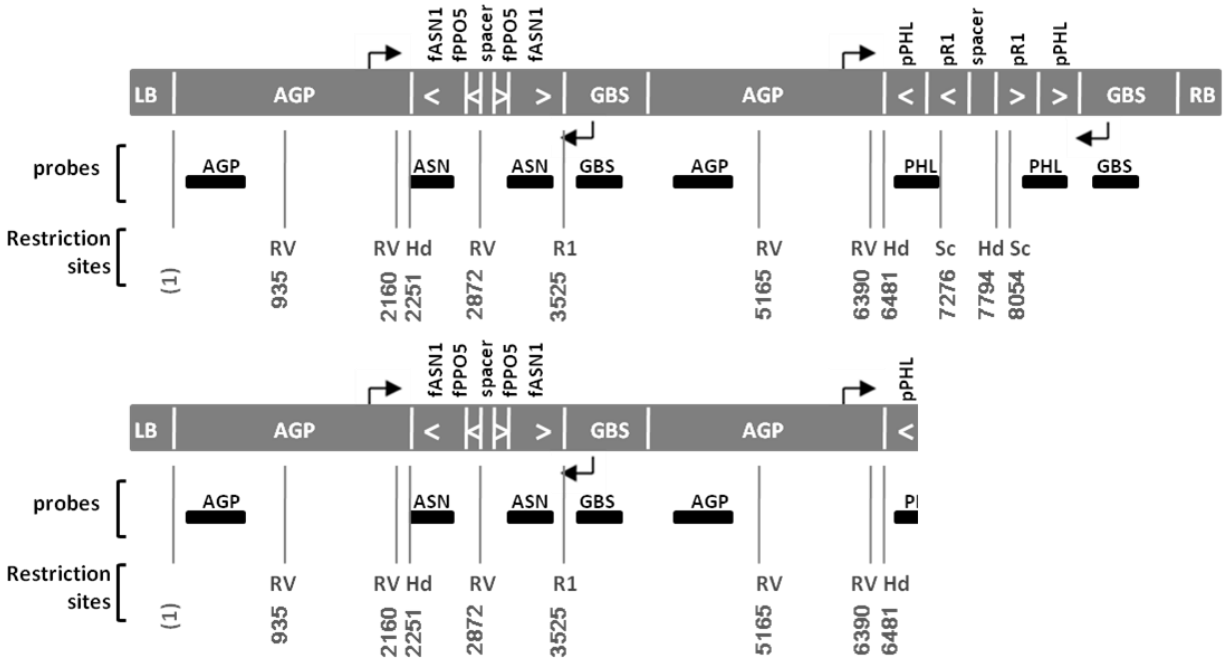
5. Variation between expected and observed sizes in DNA insert.
Slight differences were found between the observed and expected size of a 3.8-kb internal band between restriction sites for EcoR1 at 3,525-bp and Sca1 at 7,276-bp. This band was expected with the AGP, PHL,and GBS probes with EcoR1 and Sca1, but appeared sometimes at 3.7-kb. This may be because of differences in gel loading or possibly the influence of plant material resulted in faster movement within the gel. See further discussion in section titled "Additional Studies on DNA insert Integrity for all Events."

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with "*" were double intensity. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Copy Number and Integrity: H Event H50

The hybridization data for event H50 are shown in **Figures 32-35** and summarized in **Table 13**. Event H50 contains one complete DNA insert as well as one truncated copy (see **Figure 36** below).

Figure 36. H50 Inserts



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII

The presence of two **JBs** at the left border side was observed in all the digests hybridized with the AGP probe and in the *Eco*RI/*Sca*I digest hybridized with the ASN probe. These results suggested two inserts with a Left Border side. However, all the digests probed with GBS indicated the presence of a single copy containing a Right Border side. One of the inserts is complete, because all the expected **IB**s were present and because the **JBs** identified were greater than the minimum size expected. However, no I**Bs** were observed in the other DNA insert copy when HindIII and EcoR1/ScaI digests were hybridized with PHL probe, indicating that part of pPHL/pR1 inverted repeat was deleted. This analysis was confirmed by sequencing the junction fragments (see **Appendix 4. Junction Analysis and Event-Specific Detection**).

Table 13. H Event H50: DNA fragments Hybridizing with DNA Insert-Derived Probes
(Summary of observations from **Figures 32-35**)

Probe	Restriction Enzyme	Type of Band (kb)					Interpretation
		OB	**IB**	**IB**	**JB**	**JB**	
			Exp.	**Obs.**	**Exp.**	**Obs.**	
AGP Figure 32	EcoRV	8.0 7.2 5.4 2.2 1.5	2.3	2.3*	1.1+	4.0 1.9	**1.** ***Copy number at Left Border side*** Visualization of two **JB**s in three DNA digests with the AGP probe and EcoRI/ScaI with the ASN probe indicated that H50 contained two copies of the DNA insert comprising the Left Border side. **2.** ***Copy number at Right Border side*** Visualization of a single **JB** in most digests with the PHL and GBS probes indicated that H50 contained at least one DNA insert comprising the Right Border side. The two **JB** bands observed with the HindIII digestion and PHL probe indicated a second insert copy with a truncation on the RB side. **3.** ***Internal DNA insert Integrity*** *The double intensity **IB** bands observed with the AGP, ASN and GBS probes indicated the presence of two copies of the DNA insert. However, only single intensity **IB** bands were observed with the PHL probe. This suggests that at least one copy of the DNA insert is complete as indicated by the **IBs** having the expected sizes. The second DNA insert copy has a complete cassette on the LB side (see double intensity IBs with AGP, ASN and GBS probes and EcoRVand HindIII) and truncation on the RB side (seen with the PHL probe). **4.** ***DNA insert Integrity at the extremities*** A total of four **IB and JB** bands with the AGP and ASN probes and three **IB and JB** bands with the PHL and GBS probes were found in the H50 event digests. One of the inserts is complete because the **JBs** are greater than the minimum size expected. The second insert was truncated (between the HindIII site at 6,481 and the Sca1 site at 7,276) and the deleted region included the pPHL and pR1 inverted repeat, Gbss promoter and Right Border. **5.** ***Variation between expected and observed sizes in DNA insert.*** Slight differences were found between the observed and expected size of a 3.8-kb internal band between restriction sites for EcoR1 at 3,525-bp and Sca1 at 7,276-bp. This band was expected with the AGP, PHL,and GBS probes with EcoR1 and Sca1, but appeared sometimes at 3.7-kb. This may be because of differences in gel loading or possibly the influence of plant material resulted in faster movement within the gel. See further discussion in section titled "Additional Studies on DNA insert Integrity for all Events." In the EcoR1/Sca1 digest we expected two JBs that were the same size, however there was slight variation with one of the band sizes specifically between the AGP and ASN probes.
	HindIII	12.0 8.6 7.0 6.6 6.1 5.8	4.2	4.2*	2.4+	4.7 3.6	
	EcoRI/ScaI	4.9 4.33.0 1.3 1.1	3.8	3.7	3.7+	5.7 4.3*	
ASN Figure 33	EcoRV	1.4	2.3, 0.7	2.3* 0.7#*	none	none	
	HindIII	3.4	4.2	4.2*	none	none	
	EcoRI/ScaI	9.4 8.6	none	none	3.7+	6.1 4.2	
PHL Figure 34	EcoRV	20.0 14.0 8.2	none	none	3.6+	6.9	
	HindIII	16.0 9.2 6.5	1.3	1.3	2.2+	5.7 4.3	
	EcoRI/ScaI	2.4 1.8 1.7	3.8	3.7	1.9+	3.7	
GBS Figure 35	EcoRV	12.0 8.4	2.3	2.3*	3.6+	6.9	
	HindIII	0.9	4.2	4.2*	2.2+	5.7	
	EcoRI/ScaI	4.3 3.6	3.8	3.8	1.9+	4.3 3.7	

The definition of OB, IB, and JB can be found in the **Introduction**. Bands indicated with "*" were double intensity. Bands indicated with the symbol "#" were visualized in a blot shown in **Figure 40** (for 0.7-kb IB with the ASN probe in EcoRV digestion).

Additional Studies on DNA Insert Integrity for All Events

In some cases, minor differences were observed between expected sizes of internal bands and what we observed. In multiple events, an internal band was observed of about 3.6-kb in size where we knew the expected size to be 3,751 or about 3.8-kb. This band occurs between restriction sites for EcoR1 at 3,525-bp and Sca1 at 7,276-bp and was expected to be visualized with the AGP, PHL, and GBS probes with EcoR1 and Sca1 digests. The difference of about 0.15-kb between observed and expected sizes most likely was related to small differences in gel loading or the influence of plant tissues that would not be part of the marker lanes. The largest difference between expected and observed values was found for events E12 and E24. The same sequence of DNA insert containing the second AGP promoter (expected size was 3.8-kb with EcoR1 and ScaI) was studied using the HindIII digests. For example, expected bands with the AGP, ASN, and GBS probes of 4.2-kb between 2,251 and 6,481-bp all include the AGP promoter region. For all three probes, the observed bands matched the size of the expected bands for both events E12 and E24 (Tables 6 and 7). In addition, the 1.3-kb band between 6,481 and 7,794-bp restriction sites for HindIII, was observed with the PHL probe in both E12 and E24. Thus, additional probes and restriction digests support the observation that the DNA insert in the region of the second APG promoter was intact.

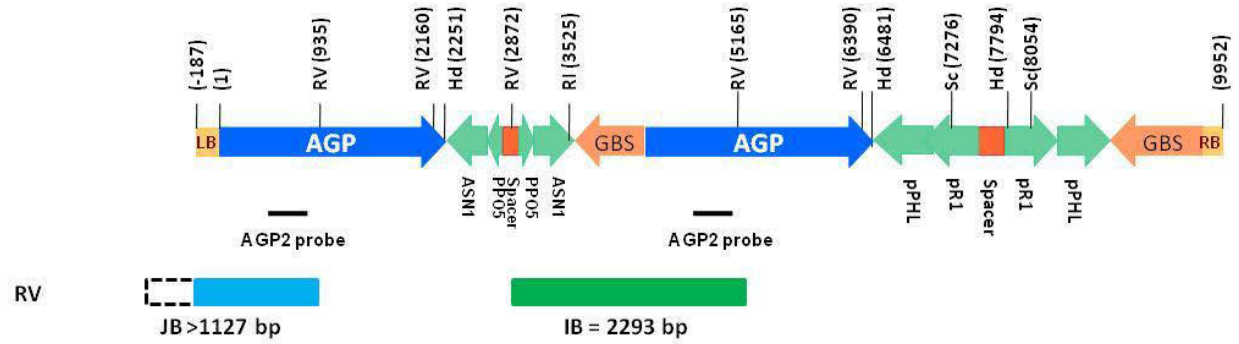
To investigate the possibility of a truncation, we conducted studies with probes that would anneal to the AGP promoter in that region. Further evidence for the structure of the various inserts was obtained by performing additional studies using a new probe that was designed to anneal to the downstream part of the AGP promoter. This 402-bp probe, AGP2, was amplified with primers HY1659 and HY1660 (**Figure 37 and Table 14** in **Materials and Methods**). As shown in **Figure 38**, hybridization with AGP2 visualized an expected 2.3-kb band in DNA of all events, indicating that no AGP promoter residing within the DNA insert was truncated in this region. A 999-bp probe, AGP3 (**Figure 39**), was amplified with primer HY1661 and HY2662 (**Figure 37 and Table 14** in **Materials and Methods**) and specifically targeted the AGP promoter region between the two *EcoR*V digests with an expected size of 1,225-bp. As shown in **Figure 39**, the J55 and H37 events each had extra hybridizing DNA fragments in addition to the 1,225-bp fragment. This indicated that an AGP promoter residing within one of the DNA inserts of these events was truncated at this range.

A short electrophoresis run of *Eco*RV digests probed with ASN confirmed the presence of the relatively small internal *Eco*RV fragments with an expected size of 0.7-kb (**Figure 40**). The presence of this band provides additional proof of the integrity of the DNA inserts.

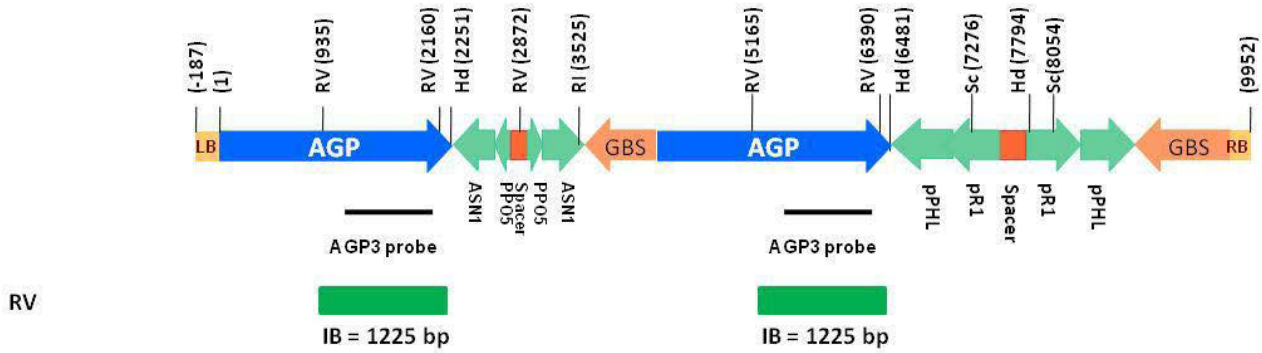
Even more evidence exists for the completeness of the DNA insert in the region of the AGP promoter (3,525 to 7,276-bp) in the gels used for stability testing (See **Appendix 3, Evidence for stability of the inserted DNA**). For events F10 and F37 (**Evidence for stability of the inserted DNA : Figures 4, 5, and 6**), the gel shows the 2.3-kb section of DNA insert that hybridizes with the AGP probe between the EcoRV restriction sites at 2,872 and 5,165-bp. This same 2.3-kb band is visualized for events E12 and E24 (Evidence **for stability of the inserted DNA : Figures 11, 12, and 13**), J3, J5, and J78 (**Figures 18, 19, and 20**), G11 (**Figures 25, 26, and 27**) and H37 and H50 (**Figures 33, 34, and 35**). Thus, the stability studies provide additional evidence that the DNA insert is intact in this region.

Figure 37. Expected IB and JB of DNA Insert Digested with Various Enzymes and Hybridized with (A) AGP2 and (B) AGP3 probes.

(A)

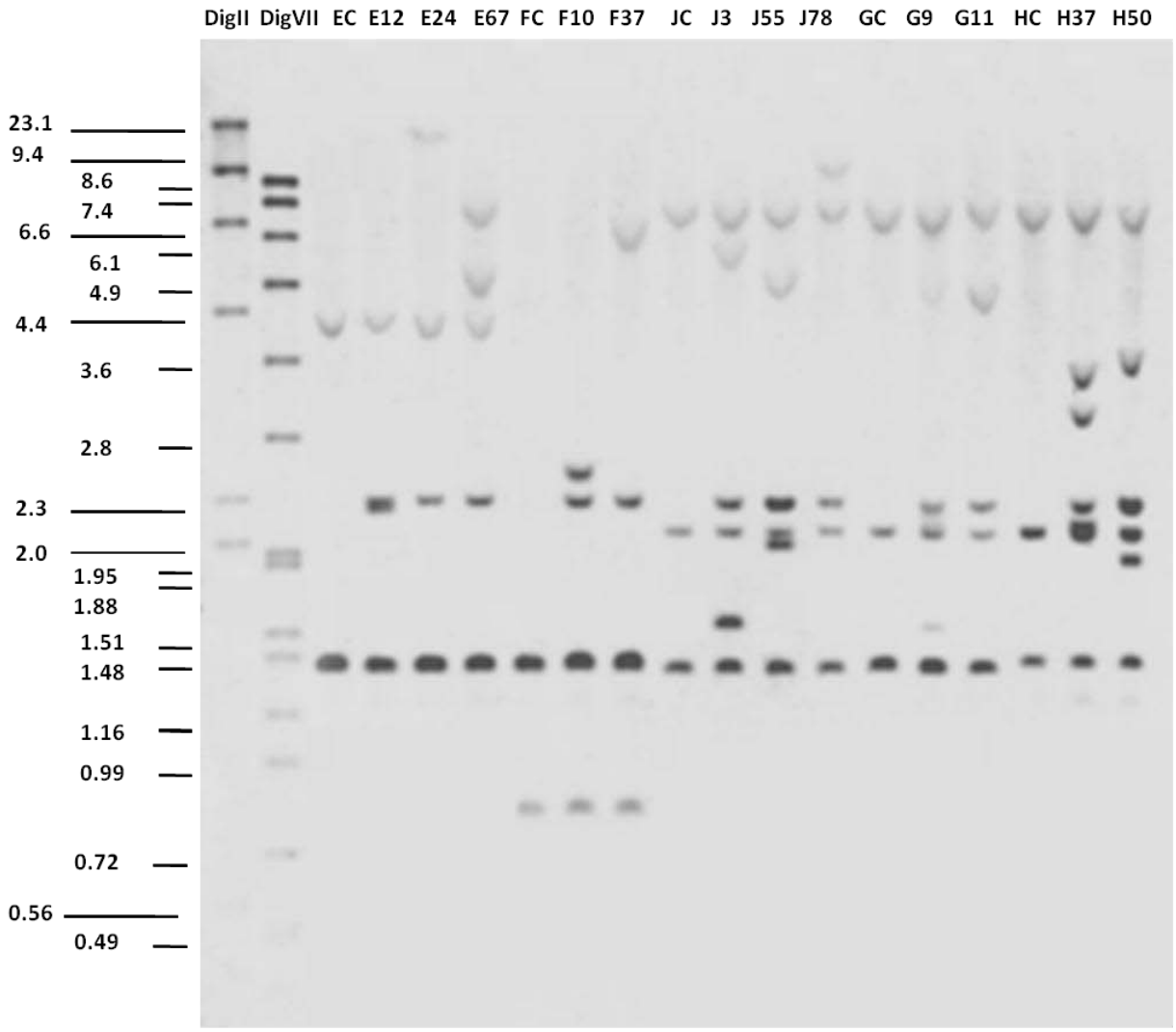


(B)



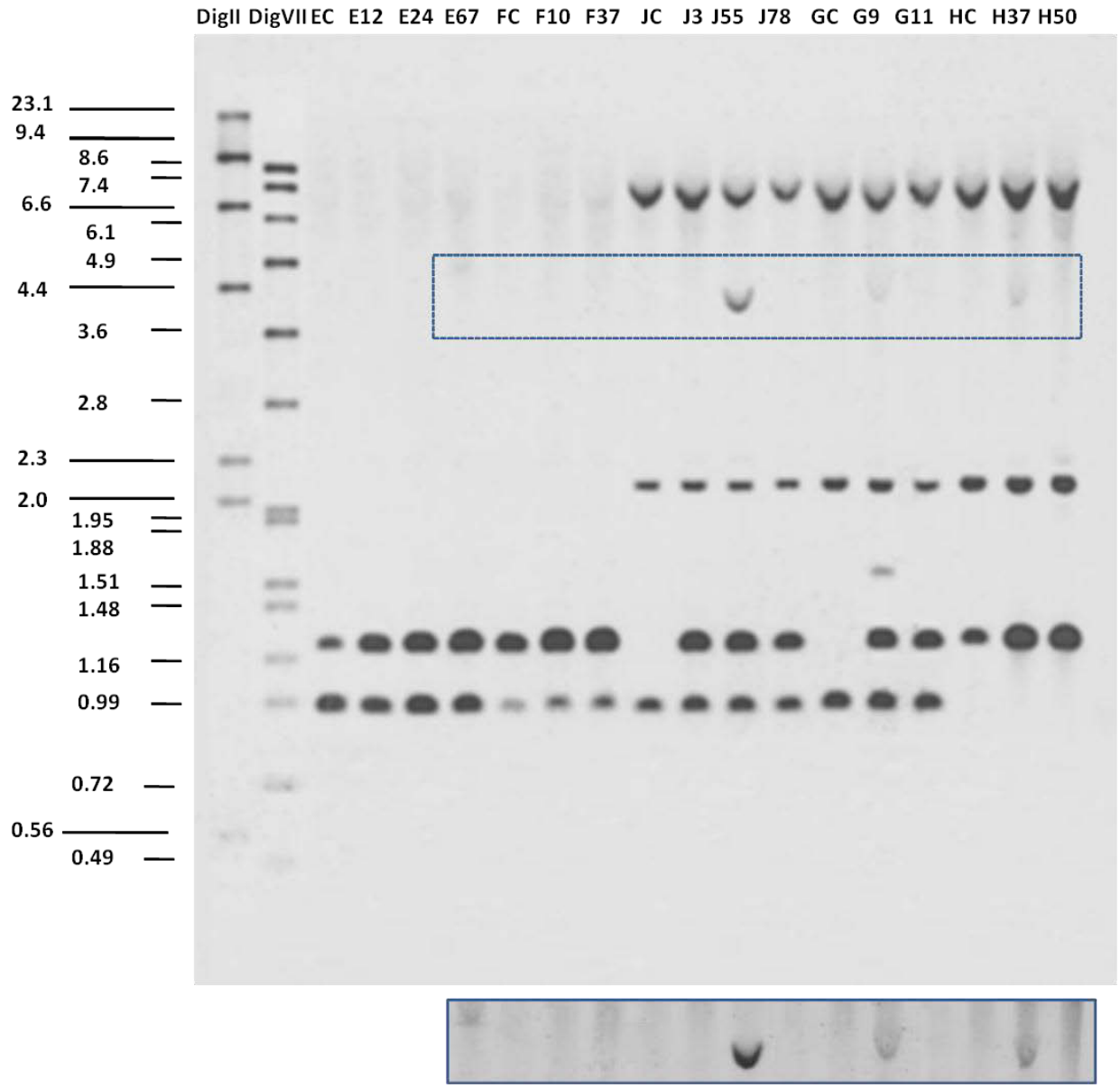
RV=EcoRV
LB = Left Border like region containing 25-bp Left Border and 162-bp flanking sequence.
RB = Right Border like region containing 25-bp Right Border and 161-bp flanking sequence.

Figure 38. Hybridization of EcoRV Digests with AGP2 Probe



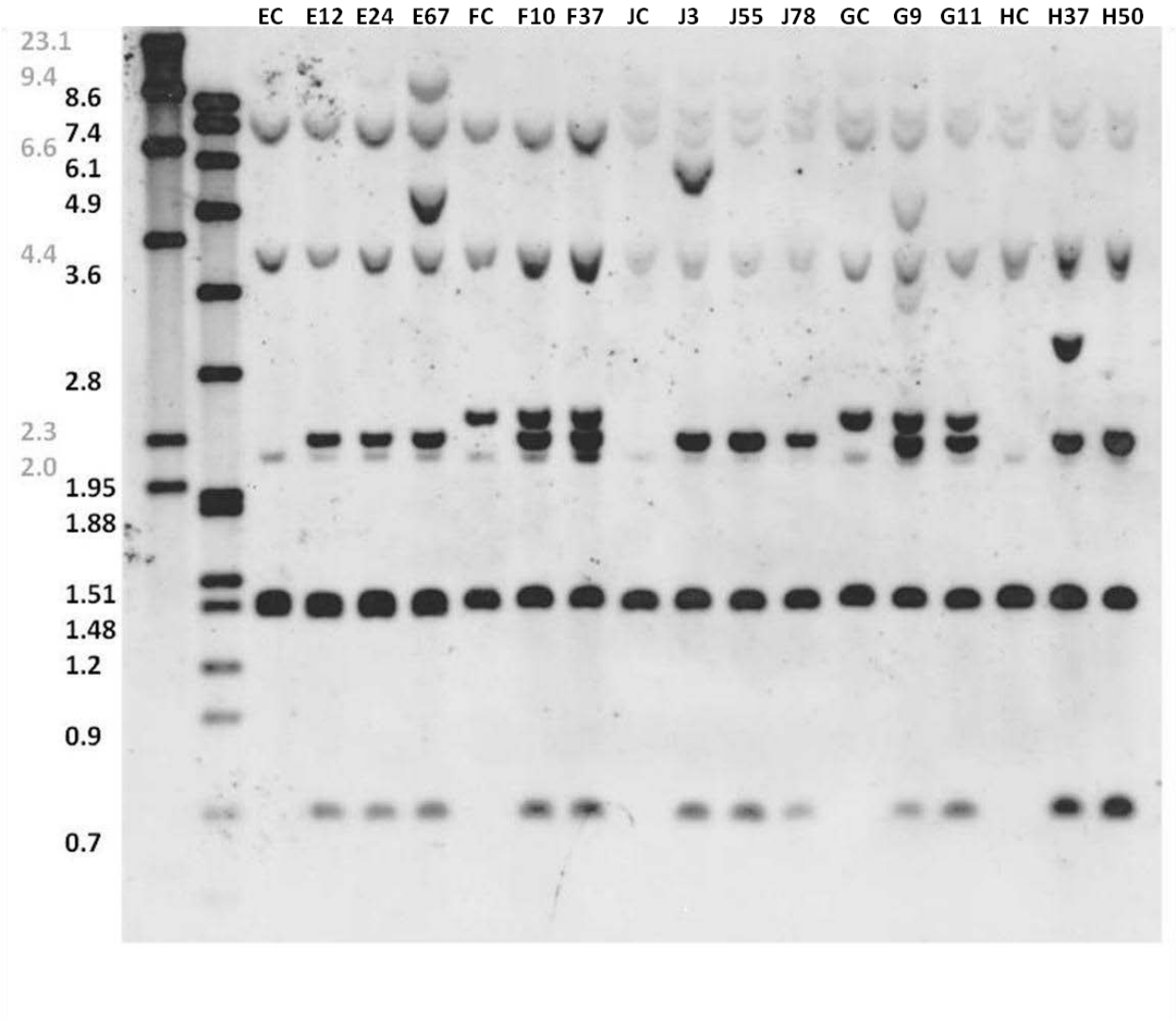
Genomic DNA was digested with EcoRV and hybridized with the AGP2 probe. Samples were loaded following the variety order E (Russet Burbank), F (Ranger Russet), J (Atlantic), G (G) and H (H). EC, FC, JC, GC and HC are wild type control samples for each variety. Size of the DigII and Dig VII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Events G9 and E67 are not part of this submission.

Figure 39. Hybridization of EcoRV Digests with AGP3 Probe



Genomic DNA was digested with EcoRV and hybridized with the AGP3 probe. Samples were loaded following the variety order E (Russet Burbank), F (Ranger Russet), J (Atlantic), G (G) and H (H). EC, FC, JC, GC and HC are conventional variety control samples for each variety. Size of the DigII and Dig VII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). A boxed segment of the blot is shown again below the blot with different brightness and contrast to highlight the 4.2-kb bands in J55, G9, and H37. Events G9 and E67 are not part of this submission.

Figure 40. Visualization of an Expected 0.7-kb Band in All Events when Hybridized with the ASN Probe
(in addition to previously observed bands in shorter runs)



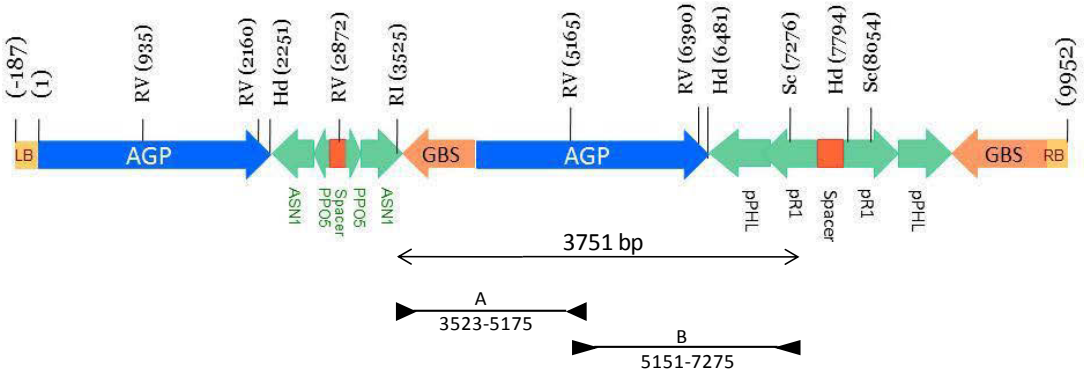
Genomic DNA was digested with EcoRV and hybridized with the ASN probe. Samples were loaded following the variety order E (Russet Burbank), F (Ranger Russet), J (Atlantic), G (G) and H (H). EC, FC, JC, GC and HC are wild type control samples for each variety. Size of the DigII and Dig VII molecular weight markers are indicated adjacent to the blot image in kilobase pairs (kb). Events G9 and E67 are not part of this submission.

PCR Confirmation of EcoR1/ScaI Fragments in E, F J, and H lines

Minor differences were observed in some cases between expected sizes of internal bands and what we actually observed. The description and figures below clarify why we observed the minor differences.

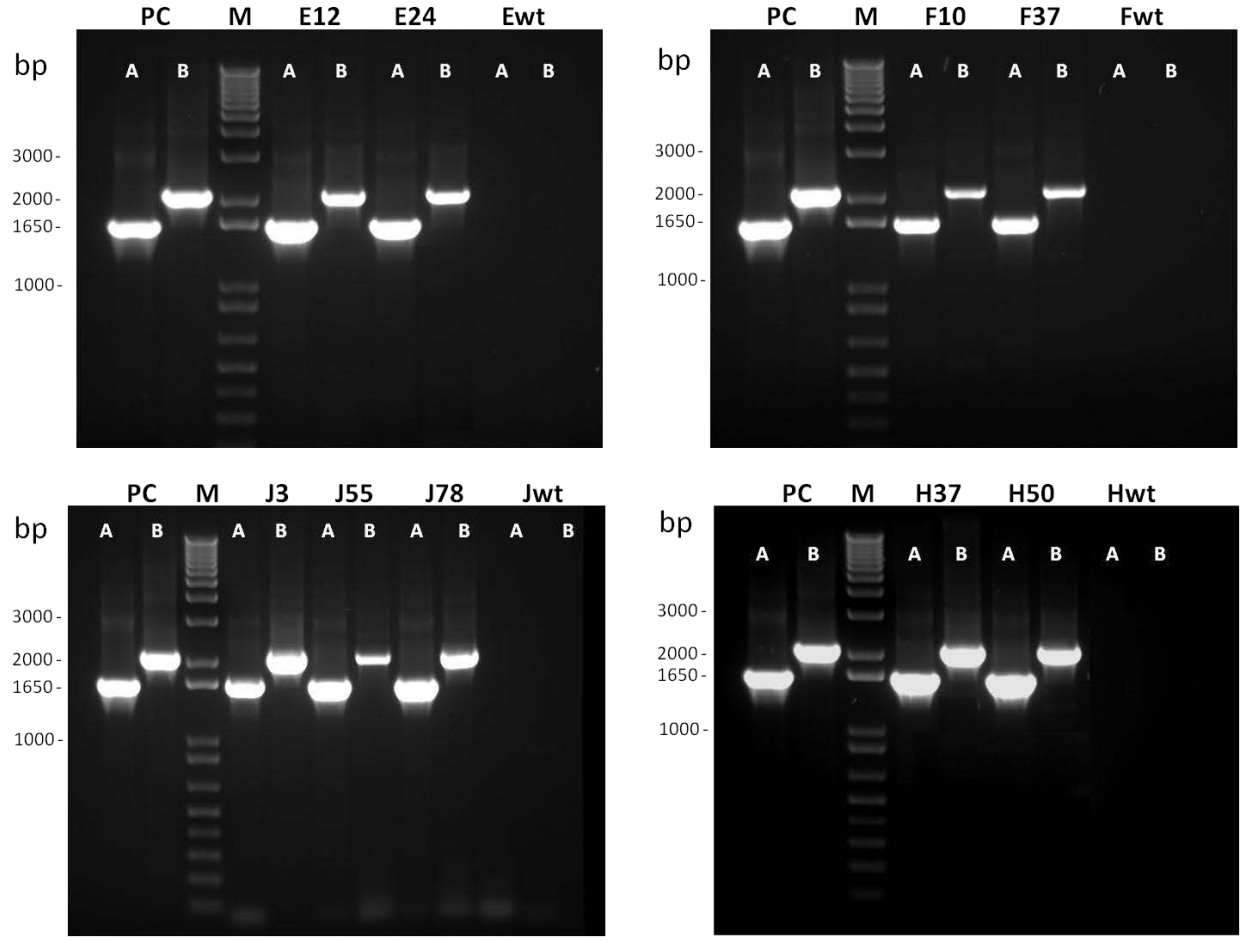
When the plant genomic DNA is digested with EcoR1 and ScaI a 3751-bp internal fragment of the DNA insert is expected in all events except G11. In addition to Southern blot analysis, a PCR approach was used to further ensure the completeness of the fragment. As shown in **Figure 41**, four primers were designed to amplify two fragments: (A) a 1653-bp fragment including EcoR1 site (3525) and EcoRV site (5165); (B) a 2125-bp fragment from EcoRV site (5165) to ScaI site (7276). Since the component targeted by a forward primer was different from one targeted by a reversed primer, no fragments were expected to be amplified from untransformed parent controls. The pSIM1278 plasmid DNA was also used as a positive control to amplify the fragment A and B. The results were shown in **Figure 42**. Both fragment A and fragment B were amplified from transformed events but not from untransformed controls. The sizes of the two fragments are the same as the expected and the positive controls. We can conclude that the 3751-bp internal fragment of DNA insert exists in events E12, E24, F10, F37, J3, J55, J78, H37, and H50.

Figure 41. The primers and PCR fragments to confirm 3751-bp EcoR1/ScaI fragment of DNA insert



Fragment	Forward primer	Reverse primer	Length
A	CGAATTCGTGATGTGTGGTC	GGATGTATGATATCACATGTGTTTG	1653 bp
B	CAAACACATGTGATATCATACATCC	ACTATTGCAAGAAAATATCAAAGGC	2125 bp

Figure 42. Confirmation of a 3751-bp internal fragment of DNA insert in E, F, J, and H lines



Bands on the ethidium bromide-stained gels are indicative for the presence of fragment A and B. Data for all events, except for G11, are shown. M = one kb DNA marker lane (invitrogen), PC = positive control, wt = untransformed recipient variety ("wild type", the negative control).

SUMMARY

Events F10, F37, E12, and E24 contain a single DNA insert comprising all sequences needed for expression of both the *Asn1*/*Ppo5* gene silencing cassette and R1/PhL promoter silencing cassette.

Events J78 and G11 contain a single DNA insert comprising the sequences needed for expression of the *Asn1*/*Ppo5* gene silencing cassette. These two events do not contain all sequences required for expression of the R1/PhL promoter silencing cassette.

Events J3, J55, and H50 contain 2 copies of the DNA insert, one of which is full and the other one lacking at least part of the R1/PhL promoter silencing cassette.

Event H37 contains one full copy of the DNA insert and three additional DNA insert fragments.

MATERIALS AND METHODS

Plant Material. Plants used for DNA gel blot analysis were grown for two months in Sunshine mix-1 (www.sungro.com) in two-gallon pots in a greenhouse that was controlled for temperature (18°C minimum/27°C maximum) and light (16-h photoperiod with an intensity of ~1500 µmol/m2/s).

DNA Isolation. For plant DNA isolation, 1.0 g of young potato leaves was ground into a fine powder in a mortar using 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 until ready to process. 10 ml extraction buffer (0.35 M Sorbitol, 0.1 M Tris-HCl, pH8.0, 0.05M EDTA) was added to, and mixed with, the powder, and the resulting suspension was centrifuged at 3,000 rpm for 15 min at room temperature. The pellet was thoroughly re-suspended in 2 ml extraction buffer containing 200 µg RNase A. After incubating the resuspended DNA at 65°C for 20 min with 2 ml of nuclear lysis buffer (0.2 M Tris-HCl pH7.5, 0.005 M EDTA pH 8.0 and 20 mg/ml CTAB Hexadecyl Trimetyl Ammonium Bromide) and 800 µl of 5% Sarcosyl, it was mixed with an equal volume of chloroform : isoamyl alcohol (24:1), vortexed for about 1 min, and centrifuged at 3000 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 a spectrophotometer at an OD of 260 nm, whereas 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. In some cases, an alternative method was used to isolate DNA. The ground tissue from 0.7 g young leaves was mixed with 7 ml CTAB buffer (2% CTAB, 1.4 M NaCl, 0.1 M pH 8.0 Tris-HCl, 20 mM pH8.0 EDTA, 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 3000 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 dried in air and dissolved in TE buffer. The quality and concentration of DNA was determined spectrophotometrically and by running a sample on a 0.8% agarose gel. Control and event DNA was always extracted with the same method for a specific comparison.

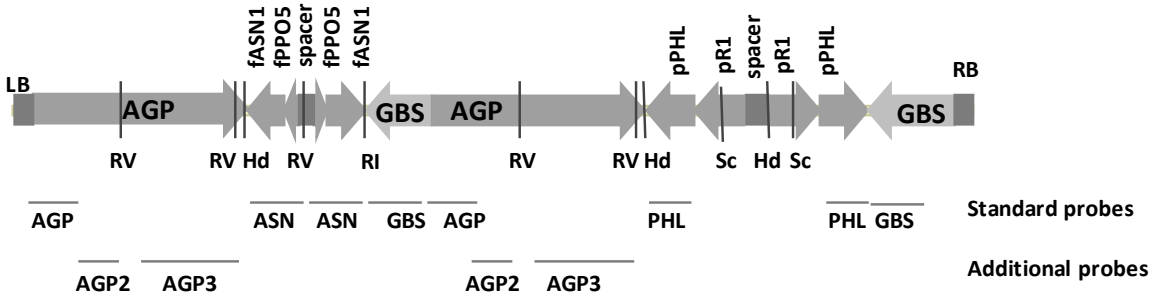
DNA Gel (Southern) Blot Analysis. 10 µg of plant DNA was digested overnight in 400 µl final volume with at least 5 µl (10 units/µl) restriction enzyme (Invitrogen) at 37°C. Digested DNA was concentrated by ethanol precipitation (40 µl of 3M NaOAc, pH5.3 and 1 ml ethanol) at -80°C for 10 min followed 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. Digested plant DNA was loaded on a large 0.7% agarose gel (170 ml) containing 0.5X tris-borate-EDTA (TBE) buffer and 3-5 µl ethidium bromide (10 mg/ml) and run at 25 volts for 18 hrs. The gel was photographed using a gel documentation system from Alpha Innotech (Santa Clara, California), and then depurinated by submerging it into 0.25 N HCl for 20 min. After subsequent denaturing in 0.5 M NaOH /1.5 M NaCl for 2X15 min and neutralization in 1.5 M NaCl and 0.5 M Tris-HCl, pH7.5, for 2 X 15 min on a shaker at room temperature and 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.

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 10 x Hotmaster Taq Buffer, 2-5 µl 10 uM forward primer, 2-5 µl 10 µM reverse primer, 5 µl DIG labeled dNTP (Roche), 10 ng plasmid template, 0.75 µl Hotmaster Taq polymerase, and

water. 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 (it always ran slower than control PCR product). The probe was denatured before use by incubating the probe at 100°C for 5 min, placing on ice for 2 min. For the exact position of probes in the DNA insert, see **Figure 43**.

Figure 43. DNA Insert Probes



RV=EcoRV, R1=EcoR1, Sc=Sca1, Hd=HindIII
LB = Left Border like region containing 25-bp Left Border and 162-bp flanking sequence.
RB = Right Border like region containing 25-bp Right Border and 161-bp flanking sequence.

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 hrs in a bottle in a standard hybridization oven (Amerex Instruments Inc.) at 20-25 rpm. Hybridization was carried out by replacing the prehybridization buffer with a fresh amount of the same preheated solution, now containing 25-50 µl denatured DIG labeled probe, and continuing the incubation at 42°C, 20-25 rpm for about 16 hrs. The hybridization solution could be store at -20°C and reused up to 3 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 brief 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 hrs 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 a plastic film and exposed to the Z-ray film in the dark. Depending on the experiment, multiple exposures were taken from 30 sec to 30 min. The films were developed with Konica SRX-101A Z-ray film developer. The developed films were scanned to obtain the final images.

Explanation of Bands Visualized by DNA Gel Blot Analysis. DNA gel blot analysis was performed using DNA extracted from greenhouse-grown plants and digested with the following restriction enzymes: (1) *Eco*RV, (2) *Hin*dIII, and (3) *Eco*RI + *Sca*I. The positions of the restriction sites and probes employed are shown in **Figure 43**. The digested DNA was run with standard DIG-labeled molecular weight markers, DIGII and DIGVII, through 0.7% agarose gels in 0.5X TBE buffer and blotted onto Nylon Hybond N+ filters. The four DNA insert-derived probes used were AGP, PHL, ASN, and GBS (see **Table 14**). Additional probes used were AGP2 and AGP3. Each of the original films from hybridization experiments contained, apart from the 2 markers, three DNA digests of the untransformed control and the events for one variety. To facilitate interpretations, the original films were separated into three separate segments (one for each digest) with the marker lanes copied and boxed.

When reading the bands sizes in DNA gel blots, such observations are subject to interpretation by the reviewer and slight variation could occur between the expected and observed sizes. The observed sizes could be influenced by many factors including the sample matrix or slight differences in gel running conditions. Therefore, other information was considered, including the expected sizes and observations from other gels and digests when reporting the sizes of the bands.

Table 14. Molecular Probes

Probe (bp)	Primer name	Primer sequence	Genetic Elements	Position in pSIM1278	Size of hybridizing bands		
					*Eco*RV	*Hin*dIII	*Eco*RI-*Sca*I
AGP (511)	AGP3F AGP3R	CCAAAACTGAAAGTGGGAAACC ACAACAACCCCCTCCTTGTG	AGP promoter fragment	17-527 4,247-4,757	OB: several IB: 2,293 JB: 1,127+	OB: several IB: 4230 JB: 2443+	OB: several IB: 3751 JB: 3717+
ASN (549)	HD076F2 HD076R2	GAACTCTTTATCCAGAAATG TAGTCTCTATTGAATCTGCT	ASN1 and PPO5 gene fragments	2,262-2,810 2,974-3,523	OB: several IB: 712, 2293 JB: none	OB: several IB: 4230 JB: none	OB: several IB: none JB: 3717+
PHL (436)	PhoLF PhoLR	AAAGTGTGAAGGGTATTTTTGTAAAC CGAATGAGAGTGATAAGAGAGTGAGG	PHL promoter fragment	6,518-6,953 8,376-8,811	OB: several IB: none JB: 3567+	OB: several IB: 1313 JB: 2163+	OB: several IB: 3751 JB: 1903+
GBS (546)	HD077F HD077R	TGTGGTCTACAAAAAGGGGAAT GGTAGCGGTAGGAGGGAGTT	Gbss promoter fragment	3,536-4,081 8,845-9,390	OB: several IB: 2293 JB: 3567+	OB: several IB: 4230 JB: 2163+	OB: several IB: 3751 JB: 1903+
AGP2 (402)	HY1659 HY1660	ACAAGGAGGGGGTTGTTGTACT CTTGATGTAAACACCGAATACGA	AGP promoter fragment	509-910 4,739-5,140	OB: several IB: 2,293 JB: 1,127+		
AGP3 (999)	HY1661 HY1662	TAAATTGGACGGACGATGAAAT CGGAATTTACGAGAGGACCATA	AGP promoter fragment	1,130-2,128 5,360-6,358	OB: several IB: 1, 225, 1,225 JB: none		

PCR Confirmation of EcoR1/ScaI Fragments in E, F, J, and H lines. The genomic DNA was isolated from the leaves of events E12, E24, F10, F37, J3, J55, J78, H37, and H50. The primers used for the confirmation of EcoR1/ScaI fragments in E, F, J, and H lines were described in **Figure 41**. The PCR was carried out in 30 µl of PCR reaction mixture consisting of 3 µl of 10X PCR buffer, 0.6 µl of 10 mM dNTP, 0.6 µl of 10 µM forward primer, 0.6 µl of 10 µM reverse primer, 100 ng of genomic DNA template, and 0.2 µl of HotMaster Taq polymerase (Fisher BioReagents). The PCR amplification conditions were as follows: 1 cycle of 3 min at 95 °C followed by 40 cycles of 30 sec at 94 °C, 30 sec at 55°C, 2 min at 68 °C, and finishing with 10 min at 68 °C.

CONCLUSIONS

Event Ranger Russet F10 contains a single copy of the DNA insert. As shown in **Appendix 6. Field Performance and Tuber Evaluations** and **Appendix 9. Compositional Analyses,** this insertion is not linked to any unexpected event-specific agronomic or biochemical changes.

Event Ranger Russet F37 contains a single copy of the DNA insert. This insertion is not linked to any unexpected event-specific agronomic or biochemical changes.

Event Russet Burbank E12 contains a single copy of the DNA insert. This insertion is not linked to any unexpected event-specific agronomic or biochemical changes.

Event Russet Burbank E24 contains a single copy of the DNA insert with the exception of a 0.5-kb deletion at the Left Border side. This deletion does not affect construct efficacy. This insertion is not linked to any unexpected event-specific agronomic or biochemical changes.

Event Atlantic J3 contains one nearly intact copy of DNA insert with a small deletion at the Left Border site. Attached to this first copy is a second copy in a head-to-head orientation, whereby the second copy also had a small deletion at the Left Border site and a much larger deletion, comprising the R1/PhL promoter silencing cassette, at the Right Border site. This insert arrangement resulted in a junction between part of the Agp promoter and flanking plant DNA that is unlikely to result in expression of a new open reading frame because the partial Agp promoter lacks the TATA-box, CCAAT-box, and additional 5' elements needed to induce transcription. The insertions are not linked to any unexpected event-specific agronomic or biochemical changes.

Event Atlantic J55 contains one full copy of the DNA insert and an additional truncated copy lacking the R1/PhL promoter silencing cassette. The two copies integrated at the same site as an inverted repeat. The insertions are not linked to any unexpected event-specific agronomic or biochemical changes.

Event Atlantic J78 contains a single truncated DNA insert. The insert has a small deletion at the Left Border that did not affect efficacy of the associated *Asn1/Ppo5* gene silencing cassette. A larger deletion occurred at the Right Border site that included one of the promoters associated with the R1/PhL promoter silencing cassette, and loss of efficacy of that cassette. This insertion is not linked to any unexpected event-specific agronomic or biochemical changes.

Event G G11 contains a single DNA insert insert with a large deletion at the Right Border site that includes the R1/PhL silencing cassette and results in a junction between the Agp promoter and flanking plant DNA. This flanking DNA does not contain known genes or new ORFs in the vicinity of the Agp promoter (as deduced by BLAST analysis of a 2.5-kb flanking DNA fragment, see **Appendix 4. Junction Analysis and Event-Specific Detection**, indicating that it is unlikely for the insert to have altered the expression pattern of one or more plant genes. This insertion is not linked to any unexpected event-specific agronomic or biochemical changes.

Event H H37 contains one complete DNA insert insert and three DNA insert fragments. One of the inserted fragments consists of a small part of the Agp promoter of the DNA insert. This particular insertion may have resulted in the operable fusion of a promoter and open reading frame. However, if such a fusion did occur, it neither affected plant agronomics nor tuber biochemistry. The second truncated fragment lacks the *Asn1/Ppo5* gene silencing cassette except for the Gbss promoter. The

remaining Gbss promoter is not functionally active because it lacks the 5’ sequences carrying CCAAT and TATA boxes. The fourth small fragment consists of a small part of the Agp promoter that is, for the same reasons, not functionally active. The insertions are not linked to any unexpected event-specific agronomic or biochemical changes.

Event H H50 contains one full copy of the DNA insert and an additional truncated copy lacking the R1/PhL promoter silencing cassette. The junction created by insertion of the truncated DNA insert results in fusion of the Agp promoter with flanking plant DNA. It is possible that this fusion could result in the expression of an open reading frame. However, if such expression did occur, it neither affected plant agronomics nor tuber biochemistry. The insertions are not linked to any unexpected event-specific agronomic or biochemical changes.

REFERENCES

Chawla R, Shakya R, Rommens CM (2012) Tuber specific silencing of asparagine synthetase-1 reduces the acrylamide-forming potential of potatoes grown in the field without affecting tuber shape and yield. Plant Biotechnol J, 10: 913-924.

Van Haaren MJJ, Sedee NJA, de Boer HA, Schilperoort RA, Hooykaas PJJ (1989) Mutational analysis of the conserved domains of a T-region border repeat of Agrobacterium tumefaciens. Plant Mol Bio, 13: 523-531.

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.

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Petition for Determination of Nonregulated Status for Innate™ Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise: Events E12 and E24 (Russet Burbank); F10 and F37 (Ranger Russet); J3, J55, and J78 (Atlantic); G11 (G); H37and H50 (H)

We submit this petition under 7 CFR 340.6 to request that the Administrator make a determination that the articles should not be regulated under 7 CFR Part 340.

Submitted by Pete Clark Ph.D.
J.R. Simplot Company

Prepared by Pete Clark Ph.D.* and Susan Collinge Ph.D.
J.R. Simplot Company

Submitted on March 1, 2013
J.R. Simplot Company Petition JRS01

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SPS-ØØJ55-2
SPS-ØØJ78-7
SPS-ØØG11-9
SPS-ØØH37-9
SPS-ØØH50-4

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This Document (Including Appendices) Does Not Contain CBI

Petition for Determination of Nonregulated Status

The undersigned submit this petition under 7 CFR 340.6 to request that the Administrator make a determination that the articles should not be regulated under 7 CFR part 340.

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Release of Information

J.R. Simplot Company is submitting the information in this assessment for review by the USDA as part of the regulatory process. By submitting this information, J.R. Simplot Company does not authorize its release to any third party except to the extent it is requested and required under the Freedom of Information Act, 5 U.S.C., paragraph 552; USDA complies with the provisions of FOIA and USDA's implementation regulations (7 CFR Part 1.4). Except in accordance with the Freedom of Information act, J.R. Simplot Company does not authorize the release, publication or other distribution of this information without J.R. Simplot Company's prior notice and consent.

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Abbreviations, Acronyms, and Definitions

Abbreviation	Definition
AGP	pAgp-derived probe used in DNA gel blot hybridization
ALA	Alanine
A. tumefaciens	*Agrobacterium tumefacians*
ARG	Arginine
ASN	Asparagine
ASN1	*Asn1* gene-derived probe used in DNA gel blot hybridization
Asn1	Asparagine synthetase-1 gene
ASP	Aspartic acid
Backbone DNA	DNA associated with vector backbone
chs	Chalcone synthase gene
cwt/A	Unit of measure equal to 100lbs/ acre
CYS	Cysteine
DNA	Deoxyribonucleic acid
DNA insert	The DNA sequence from pSIM1278 located between the LB and RB intended to be integrated into the potato genome
EB	Ethidium bromide
fASN1	Fragment of the *Asn1* gene
FDA	Food & Drug Administration
G0	First generation greenhouse-grown tuber seed
G1	First generation field-grown tuber seed
G2	Second generation field-grown tuber seed
G3	Third generation field-grown tuber seed
GBS	pGbss-derived probe used in DNA gel blot hybridization
GLN	Glutamine
GLU	Glutamic acid
GLY	Glycine
GM	Genetically modified
gus	β-glucuronidase gene
Hd	Restriction enzyme *Hin*d III
HIS	Histidine
IPD	Inter-genebank Potato Database
ipt	Isopentyltransferase gene – produces cytokinin hormones associated with plant growth and development
ILE	Isoleucine
LB	Left Border (a 25-base pair sequence) similar to *A. tumefaciens* T-DNA border
Left Border side	The region of the DNA insert in the vicinity of the Left Border, which may either extend up to the cleavage site in the Left Border or be truncated at a position of between a single base pair and 100s of base pairs downstream from that cleavage site
Left Border site	The 25-base pair sequence defined as Left Border, similar to *A. tumefaciens* T-DNA

Abbreviations, Acronyms, and Definitions (Continued)

Abbreviation	Definition
LEU	Leucine
LYS	Lysine
MAFF	Japan Ministry of Agriculture, Forestry, and Fisheries
MET	Methionine
MHLW	Japan Ministry of Health, Labor and Welfare
Non-coding DNA	DNA not coding for RNA that is translated into protein
ORFs	Open reading frames
pAgp	Promoter of the ADP glucose pyrophosphorylase gene
PCR	Polymerase chain reaction
pGbss	Promoter of the granule-bound starch synthase gene
PHE	Phenylalanine
PhL	Phospholrylase-L gene
PPO	*Ppo5* gene-derived probe used in DNA gel blot hybridization
Ppo5	Polyphenol oxidase-5 gene
Pro	Functionally-active promoter
PRO	Proline
R1	Water dikinase R1 gene
RB	Right Border (a 25-base pair sequence) similar to *A. tumefaciens* T-DNA border
Right Border side	The region of the DNA insert in the vicinity of the Right Border, which may either extend up to the cleavage site in the Right Border or be truncated at a position of between a single base pair and 100s of base pairs upstream from that cleavage site
Right Border site	The 25-base pair sequence defined as Right Border, similar to *A. tumefaciens* T-DNA
RNA	Ribonucleic acid
RNAi	RNA interference
SER	Serine
Sol t 1	42 kDa allergen known as patatin
Somaclonal variation	Genetic and/ or phenotypic variation among clonally propagated plants of a single donor clone; generated by tissue culture and other forms of vegetative propagation
TPS	True potato seed
T-DNA	Transfer DNA from *A. tumefaciens* delineated by left and right border sequences
THR	Threonine
TRP	Tryptophan
TYR	Tyrosine
USDA-APHIS	United States Department of Agriculture-Animal and Plant Health Inspection Service
VAL	Valine

Summary

The J. R. Simplot Company has pioneered a new approach that marks a significant and vital advance in plant breeding. Simplot's Innate™ technologies allow us to transform plants with plant DNA . In the present petition, Innate™ technologies have been used to transform potato plants with non-coding potato DNA (DNA not coding for RNA that is translated into protein). We seek nonregulated status of potato plants transformed using our Innate™ technologies and submit the evidence that these plants should not be classified as "Regulated articles" as defined under 7 CFR 340.

To explain, Simplot's Innate™ technologies allow researchers to isolate genetic elements from any plant genome, rearrange them, or link them together in desired permutations, and introduce them back into the genome. More specifically, in these events, the genomic DNA comes from potato or wild potato (a group of related plant species that are sexually-compatible with potato). We incorporate no viral markers, and no vector backbone sequences, into the plant genome.

As demonstrated in the data presented in this petition, the transformed cultivated potato plants are not parasitic, and our data show no increase in pest potential compared to the controls. Furthermore, the expression of the integrated genetic elements results in traits highly sought-after in the potato industry, namely: reduced black spot bruise, low asparagine, and lower levels of reducing sugars. One key consequence of these beneficial traits is that the harvested potatoes contain less acrylamide when cooked than untransformed, commercially available potatoes.

Among other things, we have used Innate™ technologies to address two critical potato quality issues: (1) large amounts of asparagine, a non-essential free amino acid that is rapidly oxidized to form acrylamide upon frying or baking; and (2) susceptibility to enzymatic browning and discoloration, which happens when polyphenol oxidase leaks out from the damaged plastids of bruised potatoes. In the cytoplasm, the enzyme oxidizes phenols, which then rapidly polymerize to produce dark pigments. Browning is also triggered non-enzymatically as a consequence of the partial degradation of starch into glucose and fructose. When heated, these reducing sugars react with amino acids, such as asparagine, through the Maillard reaction to produce a variety of desired compounds contributing to flavor, aroma, and browning, but also acrylamide.

Because our Innate™ technologies effectively accelerate the process of conventional crossing, it allows desired changes in traditional varieties to occur much faster than is currently possible, while maintaining the desired characteristics of the original parent plant. For potatoes, Innate™ technologies are particularly attractive because potatoes are notorious for having a high degree of heterozygosity, suffering from inbreeding depression, and limited predominantly to clonal propagation. These inherent factors significantly hinder and prevent us from commercially introducing quality, sought-after traits into this valuable crop. Today's traditional breeding techniques simply result in random genomic rearrangements and trait segregation, and do not allow for the simultaneous addition of multiple desired traits.

To illustrate this point, there currently are no varieties available that produce tubers with low acrylamide potential, reduced black spot bruise and reduced sugars, while displaying all other traits important to the food industry. Therefore, instead of attempting to develop new varieties, the J. R. Simplot Company improved the quality of five existing potato varieties by transforming them with a non-coding transfer DNA that silences the genes related to expression of black spot bruise, asparagine,

and reducing sugars in tubers. Our proprietary plant expression cassettes, included in the DNA insert, are derived from the potato genome.

This DNA insert comprises two expression cassettes and was inserted into our pSIM1278 transformation vector. The first cassette comprises fragments of both the asparagine synthetase-1 gene (*Asn*1) and the polyphenol oxidase-5 gene (*Ppo5*), arranged as inverted repeats between the Agp promoter of the ADP glucose pyrophosphorylase gene (*Agp*) and the Gbss promoter of the granule-bound starch synthase gene (*Gbss*) and results in silencing of both the *Ppo5* and *Asn1* genes. The second cassette is comprised of fragments of the promoters of the starch associated gene (*R1*) and the phosphorylase-L gene (*PhL*), operably linked to the same Agp and Gbss promoters as the first cassette. The function of the second cassette is to silence the promoters of the starch associated gene (*R1*) and the phosphorylase-L gene (*PhL*). Importantly, in keeping with our Innate™ technologies, these expression cassettes contain potato genomic DNA, *e.g.* DNA from either the selected plant species or from a plant that is sexually compatible with the selected plant species.

We transformed five different varieties including Ranger Russet, Russet Burbank, Atlantic, variety G, and variety H with pSIM1278, and identified transformed shoots using polymerase chain reaction (PCR) rather than markers. We also performed numerous tests and confirmed that none of the plants contained vector backbone DNA. Transformed plants were propagated to produce the Innate™ events. Ten of these events were selected for detailed analyses: F10 and F37 for Ranger Russet, E12, and E24 for Russet Burbank, J3, J55, and J78 for Atlantic, G11 for variety G, and H37 and H50 for variety H. See **Table 1** for their OECD unique identifiers.

Table 1. Innate™ events and their OECD unique identifiers

Event number	OECD Unique Identifier
F10	SPS-∅∅F10-7
F37	SPS-∅∅F37-7
E12	SPS-∅∅E12-8
E24	SPS-∅∅E24-2
J3	SPS-∅∅∅J3-4
J55	SPS-∅∅J55-2
J78	SPS-∅∅J78-7
G11	SPS-∅∅G11-9
H37	SPS-∅∅H37-9
H50	SPS-∅∅H50-4

Extensive analyses of the various events demonstrated that the achievement of the desired modified traits was enabled by targeted gene silencing. Simplot found there to be reduced RNA (transcript) levels of the four targeted genes in tubers, which was found to be associated with:

(1) 69 – 78 percent reductions in free-asparagine;
(2) Black spot bruise tolerance; and
(3) Lower levels of reducing sugars.

Eight of the selected events contained both expression cassettes. Two events, J78 and G11, had only the silencing cassette for the *Asn1* and *Ppo5* genes integrated into their genomes and produced tubers with successfully down-regulated expression of the *Asn1* and *Ppo5* genes. The effective reduction of

asparagine found in events J78 and G11, resulting from silencing of the *Asn1* and *Ppo5* genes results in significantly reduced acrylamide (66 to 70%), making these commercially valuable potato varieties even without silencing the starch associated genes (*R1*) and phosphorylase-L (*PhL*).

Ultimately, we demonstrate that the Innate™ tubers exhibited the desired traits which can lead to lower acrylamide levels in cooked potatoes and, importantly, we showed that the transformed tubers were otherwise substantially equivalent to untransformed control tubers.

The propagation of commercial potato varieties through cloning of seed potatoes mitigates concerns about increased weediness or pest potential such as seed dispersal, survival outside of cultivation, or outcrossing. One of the most important potato varieties, Russet Burbank, produces few flowers and is male sterile. Other factors limiting outcrossing include the tendency for most fertile varieties to be self-pollinated, an inability to attract honey bees because they lack nectar, the pollen transfer range is limited to about 20 meters, and true seeds would be unlikely to grow into mature potatoes since potato seeds are not saved and propagated in the typical farming operation. If potatoes were grown from true potato seed, the offspring would be so diverse that they would not be useful as commercial potatoes. In addition, potatoes are not known to escape from commercial fields or show weediness potential. Wild potato varieties are rare in the United States and for the most part geographically isolated from commercial production areas, further reducing concerns about cross-pollination with wild species.

The modifications described in this petition were intended to enhance the quality, not agronomic characteristics, of potato by inactivating four native genes. Planting, cultivation, management and harvesting techniques were not affected by the incorporated traits. The Innate™ varieties are likely to be planted in areas that are already growing potatoes, and would not result in a significant expansion of acres. The modifications described in this petition are highly unlikely to increase the weediness or invasiveness of potato because the incorporated traits (reduced free-asparagine, black spot bruise tolerance, and reduced sugars) do not play a role in survival, dispersal, or ecological competitiveness. Field trials over multiple years with the 10 events did not provide any evidence for altered growth characteristics such as accelerated tuber sprouting, increased plant vigor, increased tuber set, or delayed senescence. No new pesticides are expressed, and no natural defense mechanisms activated, and thus modified potatoes are unlikely to display enhanced weediness or pesticidal properties. Therefore, the potential to impact insects and other non-target organisms, weed or disease susceptibility, endangered species or biodiversity is negligible for Innate™ potatoes.

We therefore seek nonregulated status for these events based on the weight of evidence demonstrating their safety when compared to untransformed potatoes. The technologies presented in this petition and the resultant transformed plants and their tuber products satisfy the requirements for nonregulated status. The transformed plants and subsequent traits described in this petition should not be considered plant pests. In conclusion, plants displaying the incorporated traits which have resulted from the addition of well characterized, non-coding regions from potato or wild potato are as safe as untransformed potatoes based on all of the data contained in this submission. The J.R. Simplot Company requests a determination from APHIS that the 10 potato events described in this petition, and any progeny derived from these events, are granted nonregulated status and would no longer be considered regulated articles under 7 CFR Part 340.

The genetic services and research and development methods and associated genetic tools, disclosed in this petition, such as expression vectors, and the transformed plants and plant cells, and methods of transformation, have been trademark-branded by Simplot as Innate™.

1. Rationale for Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise

The potato varieties were transformed using Innate™ technologies in order to address the need of the potato industry to improve quality by reducing expression of the enzyme responsible for black spot bruise and to reduce acrylamide through lowering the concentration of the reactants, namely asparagine and reducing sugars. Use of Innate™ technologies allows for transformation of potatoes using genetic material that contains only non-coding regulatory regions. The desirable traits were simultaneously incorporated into five of the most popular potato processing varieties. Such trait addition into existing varieties would not be possible to achieve through traditional breeding because potato is tetraploid, highly heterozygous and sensitive to inbreeding depression. The resulting Innate™ potatoes are not plant pests as verified by phenotype, Southern blot hybridization, and PCR.

1.1. Basis for Determination of Nonregulated Status

Part 340 Section 7 of the United States Federal Register regulates, among other things, the introduction of organisms and products altered or produced through genetic engineering that are plant pests or are believed to be plant pests. The 10 events described in this petition were transformed with DNA that does not alter the pest characteristics of the potatoes. All events were confirmed to be free of *Agrobacterium* and free of *Agrobacterium*-derived backbone DNA. In addition, the scientific evidence presented here shows that the inserted genetic material contains only non-coding regulatory regions and are as safe as untransformed potatoes.

Field evaluations demonstrated that the events displayed similar agronomic and phenotypic characteristics compared to their untransformed controls. This submission reviews the biosafety implications of all minor differences observed. The most important intentionally-incorporated trait is a reduced level in the free amino acid, asparagine in tubers. As predicted, we confirmed that this change was associated with a reduced potential to form acrylamide upon frying.

Most of the events also displayed lowered levels of the reducing sugars glucose and fructose when fresh or when analyzed at one month after harvest (**Appendix 9. Compositional Analyses**). Although reduced asparagine alone significantly decreases acrylamide formation, as shown in events J78 and G11, the lowered levels of reducing sugars further reduces acrylamide formation and limits heat-induced browning. The plant-derived transfer DNA used for transformation consisted of two inverted repeats. Each of these repeats was inserted between promoters that are predominantly active in tubers. Expression of the first inverted repeat triggered the degradation of transcripts for the asparagine synthetase-1 (*Asn*1) and the polyphenol oxidase-5 (*Ppo5*) genes. Expression of the second inverted repeat resulted in reduction in the starch associated gene (*R1*) and the phosphorylase-L gene (*PhL*) transcripts in tubers. The events presented in this submission all contained at least the fragment of the DNA insert carrying the *Asn1*/*Ppo5* gene silencing cassette.

The 10 events produced by transformation of 5 potato varieties with the specified non-coding transfer DNA are well characterized and safe as determined by agronomic and compositional evaluations, when compared to the untransformed controls.

1.2. Rationale for Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise

The Swedish National Food Administration and Stockholm University announced in April 2002, a relationship between the formation of certain blood adducts and the consumption of foods later found to contain acrylamide (NTP 2012). Other researchers also studied the mechanism for acrylamide formation in food, and the J.R. Simplot Company had also earlier predicted that acrylamide would form as a result of the well-known but complex Maillard browning reaction. Cooking or heat treating of foods that are rich in reducing sugars and amino acids may result in browning, commonly referred to as the Maillard reaction. This reaction occurs between sugars and amino acids, affecting changes in the color, flavor, functional properties and nutritional value of food (Obrien and Morrissey 1989). More specifically, we found acrylamide formed primarily when the amino acid asparagine along with reducing sugars were heated at temperatures above 120°C, as would occur during frying and baking. The biochemical basis of acrylamide formation was later published by Stadler *et al.* (2002).

Various governments responded to the news about finding acrylamide in food by providing guidance and surveys. Through one such survey, the FDA determined that potato products contribute 35% of the acrylamide exposure through diet in the US. The scientific community has since gathered additional information, including epidemiology and toxicology studies. Along with studies to mitigate the levels of acrylamide in food, the scientific community has also been working to understand the health implications of acrylamide consumption (NTP 2012).

Based on a recent notice (Federal Register 2009), FDA is considering issuing guidance for industry on the reduction of acrylamide levels in food products. The introduction of Innate™ potatoes with low acrylamide potential would provide potatoes that are largely indistinguishable from existing varieties. The reduction in asparagine and sugars using Innate™ technologies and the resulting reduction in acrylamide upon heating will address food industry needs with respect to the proposed FDA guidance. As such, this approach to lowering acrylamide could be adopted readily by the food industry if such guidance is issued by the FDA. In addition, litigation in the state of California as a result of Proposition 65 (OEHHA 2011a; OEHHA 2011b) resulted in legal settlements with restaurant chains and the retail french fry and potato chip manufacturers regarding perceived risks from acrylamide. Some settlements required signs warning consumers, but in addition, some manufacturers of retail french fries and potato chips would be required to reduce acrylamide in their products or add warning labels. As of December 1, 2011, some potato chip manufacturers began including a statement on packaging warning consumers of the presence of acrylamide and its relationship to browning in potatoes. Potato processors affected by these rulings in California could be motivated to adopt the low acrylamide, Innate™ potato products.

Following the discovery of acrylamide in foods and in response to governmental concerns, many additives were studied and some have shown effectiveness at reducing acrylamide. Some of the more promising options include citric acid, enzymes such as asparaginase, and amino acids that substitute for asparagine in the Maillard reaction and therefore form products other than acrylamide when heated.

Another approach to reducing acrylamide could be based on either traditional breeding or biotechnology to reduce the precursors for acrylamide. The use of biotechnology allows for specific deactivation of genes involved in the formation of asparagine and reducing sugars. In a crop like potatoes, which is highly heterozygous and suffers from inbreeding depression, the speed to develop such changes could take decades through traditional breeding. In contrast, biotechnology allows for specific changes in traditional varieties much faster, while maintaining the desired characteristics of the original parent.

Potato transformation with the pSIM1278 construct results in the incorporation of two silencing cassettes into the potato. Expression of the first cassette lowers transcript levels for the *Asn1* (asparagine synthetase-1) and *Ppo5* (polyphenol oxidase-5) genes and, consequently, limits (1) formation of the acrylamide precursor asparagine, and (2) formation of impact-induced black spot bruise that occurs when the enzyme polyphenol oxidase oxidizes phenols to produce dark pigments. The presence of black spot bruise results in lower quality and subsequent production losses during processing into fries or chips. A reduction in the formation of reducing sugars is accomplished by the down-regulated transcript levels for the *PhL* (phosphorylase-L) and *R1* (starch associated) genes resulting from expression of the second cassette. These traits function by slowing the conversion of starch to reducing sugars (glucose and fructose). Benefits include improved quality, especially relating to color control, and thus contributing to the desired golden brown colors required by most french fry or chip customers. Also, the reducing sugars react with amino acids, such as asparagine, to produce Maillard products including acrylamide.

1.3. Benefits of Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise

The events described here produce tubers with a greatly reduced potential to form acrylamide, thus addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

Many methods have been tested and research is ongoing to reduce acrylamide through process changes, reduction in dextrose, and additives such as asparaginase, citrate, and competing amino acids. The required capital expense to implement process changes throughout the potato industry would cost millions of dollars. In addition to the expense, these process changes have significant drawbacks including potentially negative flavors associated with additives such as asparaginase or citrate. Typically, fry manufacturers add dextrose during processing of french fries to develop the desired golden brown color, but dextrose also increases the formation of acrylamide through the Maillard reaction. Significant reductions in acrylamide occur by merely omitting dextrose from the process; however, the signature golden brown colors must then be developed some other way (such as though the addition of colors like annatto). The use of alternate colors, results in an absence of the typical flavors that develop through those browning reactions. Another challenge with the use of additives to reduce reactants like asparagine will be the principle of moisture migration that occurs during frozen storage resulting in a return of asparagine to the surface and increased acrylamide compared with the initial testing at the time of processing. In contrast, the Innate™ potatoes will significantly reduce acrylamide without the use of new additives or process changes. The resulting foods will have the same appealing color, flavors, and aromas as they currently have today.

The blackening that occurs after potatoes are bruised affects quality and recovery in processing french fries and chips. Potatoes that have been damaged and show black spot bruise must be trimmed or could be rejected before processing, resulting in quality challenges or economic loss. Management of reducing sugars presents significant challenges and potential financial loss because processors reject loads with reducing sugar content above 2%, which is approximately 20% of potatoes produced. However, potatoes developed with Innate^{TM} technologies exhibit significantly less black spot bruising and lower reducing sugar content, which will likely have economic benefits for growers and processors.

1.4. Submissions to Other Regulatory Agencies

Simplot has initiated and will complete a consultation process with the Food and Drug Administration (FDA) prior to commercial distribution of tubers or products from the 10 events.

An assessment of the safety of these events will also be submitted to Health Canada and the Canadian Food Inspection Agency (CFIA) in 2013. Furthermore, submissions in support of approval to import products from the events will be made to the Japan Ministry of Health, Labor and Welfare (MHLW) and the Ministry of Agriculture, Forestry, and Fisheries (MAFF). Submissions are anticipated for other key international markets including Mexico.

1.5. Conclusions: Rationale for Development of Potatoes with Low Acrylamide Potential and Reduced Black Spot Bruise

In summary, there is an important need to improve the quality and reduce levels of acrylamide in fried and baked potatoes. Because it was not feasible to reduce acrylamide formation and black spot bruise in existing varieties through traditional breeding, we accomplished this goal by applying Innate™ technologies. The varieties represented in this petition represent almost 30% of the US Potato market. However, about 70% of the remaining acreage and over 70 major varieties could benefit from the reduction in acrylamide through transformation with pSIM1278. We now seek nonregulated status for 10 events based on the weight of evidence demonstrating their safety. We have transformed plants with the addition of genomic DNA from potato and wild potato where the integrated genetic material contains only well characterized non-coding regulatory regions that are as safe as untransformed potatoes.

2. The Biology of Potato

This section is based, in part, on the Organization for Economic Co-Operation and Development's (OECD) series on harmonization of regulatory oversight in biotechnology no. 8, Consensus document on the biology of Solanum tuberosum, 1997.

This section describes potato biology and how it contributes to the effective management of the Innate™ potatoes. All commercial potato varieties must be propagated through cloning, effectively mitigating many concerns about increased pest potential such as seed dispersal, survival outside of cultivation, or outcrossing.

2.1. History of Potato

The cultivated potato traces its origin to Andean and Chilean landraces developed by pre-Colombian cultivators. These landraces exhibit morphological and genetic diversity, and are distributed from western Venezuela to northern Argentina, and in southern Chile (Spooner *et al.* 2005). Introduced to Europe by Spain in 1536, the potato was subsequently conveyed by European mariners to territories and ports throughout the world. Once established in Europe, the potato soon became an important food staple and field crop. But lack of genetic diversity, due to the fact that very few varieties were initially introduced, left the crop vulnerable to disease. In 1845, a plant disease known as late blight, caused by the fungus-like oomycete pathogen *Phytophthora infestans*, spread rapidly through the poorer communities of western Ireland, resulting in the crop failures that led to the Great Irish Famine. Potato remains an essential crop in Europe, where per capita production is still the highest in the world, but the

crop also made its way back over the Atlantic Ocean to North America. The industry gained greater importance particularly in western states after the horticulturist Luther Burbank developed the Russet Burbank potato in 1872. In 2011, the United States harvested 21.4 million tons of potatoes, enough to make it the world's fourth biggest producer (NPC 2012). Potatoes in the United States are grown in many states, although about half of the crop comes from Idaho and Washington. The top ten potato producing states of Idaho, Washington, Wisconsin, North Dakota, Colorado, Oregon, Maine, Minnesota, California and Michigan account for almost 88% of the United States potato crop (USDA-NASS 2010b). Most potatoes are harvested in July through October. Only about one third of US potatoes are consumed fresh. Around 60% of annual output is processed into frozen products (such as frozen fries and wedges), chips, dehydrated potato and starch, while 6% is replanted as seed potato. Americans eat, on average, approximately 54 kg of potatoes per person per year. The most rapid expansion in production and consumption of potatoes has occurred in southern and eastern Asia. China is now the world's largest potato-producing country, and nearly a third of the world's potatoes are harvested in China and India.

2.2. Use of the Potato as Food and Feed in the USA

Harvested potatoes are either used for food (~93%), feed (<1%), industrial purposes (<1%) or as “seed” for planting (5%). The type of use is partially dependent on a variety of characteristics related to tuber quality. According to the National Potato Council, approximately 50% of all tubers are used for the combination of frozen fries, potato chips, and shoestrings (julienne cut crispy snacks), whereas 11% are dehydrated and <2% are canned. An additional 28% of tubers are not processed but sold as fresh potatoes (NPC 2012).

Raw potato waste products (peels, out of specification raw potatoes, or other non-processed raw potato products) and processed discards (french fry, hash brown, etc.) are routinely incorporated into feed rations at livestock feedlot operations including those owned by the J.R. Simplot Company. Approximately 15,000 tons of processed potato waste and 60,000 tons of raw potato waste are integrated into livestock feed at the J.R. Simplot Company’s Grandview, ID feedlot on an annual basis. The typical feed ration used for cattle finishing at J.R. Simplot Company feedlots includes 8-14% potato waste. One of the major benefits of using the potato waste is that it is not discarded into landfills, but used in a sustainable manner as part of a long-term approach to reducing waste and integrating sustainability with respect to the Earth’s valuable natural resources.

2.3. Taxonomy of the Genus *Solanum*

The *Solanaceae* family contains several well-known cultivated crops such as tomato (*Solanum lycopersicum* also referred to as *Lycopersicon esculentum*), eggplant (*Solanum melogena*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*) and potato (*Solanum tuberosum*). Within the genus *Solanum*, over a thousand species have been recognized. Potatoes will not hybridize with non-tuber bearing *Solanum* (tomato, eggplant, etc.) species including weeds commonly found in and around commercial potato fields (Love 1994).

The genus *Solanum* is divided into several subsections, of which the subsection *potatoe* contains all tuber-bearing potatoes. The subsection *potatoe* is divided into series, of which *tuberosa* is relevant to this document. Within the series *tuberosa* approximately 54 species of wild and cultivated potatoes are found. One of these is *S. tuberosum*.

S. tuberosum is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum* is the cultivated potato widely in use as a crop plant in, for example, North America and Europe. The subspecies *andigena* is also a cultivated species, but cultivation is restricted to Central and South America (Hanneman 1994).

2.4. Genetics of Potato

The basic chromosome number in the genus Solanum is twelve. *S. tuberosum* subsp. *tuberosum* can be diploids (2n=2x=24) or tetraploids (2n=4x=48). The diploids have a limited range in parts of South America, while the tetraploids are the most commonly cultivated all over the world. How tetraploidy originated in potato is unclear. The cultivated *S. tuberosum* subsp. *tuberosum* can be either an autotetraploid (doubling of the chromosomes of a diploid species) or an allotetraploid (doubling of the chromosomes of a diploid hybrid between two related species).

While nearly all diploid species are self-incompatible, the cultivated tetraploid *S. tuberosum* subsp. *tuberosum* is capable of self-pollination (selfing). Plaisted (1980) has shown that under field conditions selfing is most likely for tetraploid *S. tuberosum,* with 80-100 percent of the seeds formed due to selfing. Conner and Dale (1996) collected outcrossing data from several field experiments with genetically modified potatoes, performed in New Zealand, the United Kingdom and Sweden. In each study, the outcrossing rate was zero when receiving plants were separated by more than 20 meters from the genetically modified ones. Although many Solanum species are fertile, it appears that a large number of the tetraploid cultivated *S. tuberosum* subsp. *tuberosum* cultivars have reduced fertility. Events of three of five varieties in this petition produce abundant flowers and pollen. However, Russet Burbank and the H varieties and events are sterile and have no outcrossing potential.

2.5. Potato Growth and Life Cycle

Potatoes grown in both commercial and organic farming operations go through the following five general growth stages of development (Dwelle 2003): 1) Sprouts develop from eyes on seed tubers, grow upwards, and emerge from the soil. Roots initiate development at the base of the emerging sprouts, 2) Vegetative growth begins. Leaves and branch stems develop from aboveground nodes. Roots and stolons develop at belowground nodes, 3) Tubers begin to form at stolon tips, but do not enlarge. Flowering is initiated at the end of this stage, 4) Tuber cells expand with the accumulation of water, nutrients, and carbohydrates, and 5) Vines turn yellow and lose leaves, tuber growth slows and vines die. Tuber maturation is completed as tuber skins set.

In greater detail, the potato life cycle begins with certified seed potatoes, which are cut into pieces containing at least one eye and are typically planted in the field from February through May. Plants will establish by forming vegetative tissues, including roots, leaves, stems, and stolons. Tuber formation begins approximately 30-60 days after planting. Tubers are derived from lateral underground buds that develop at the base of the main stem, when kept underground. These develop into stolons due to diagravitropical growth. When conditions are favorable for tuber initiation, the elongation of the stolon stops, and cells located in the pith and the cortex of the apical region of the stolon first enlarge and then divide longitudinally. The combination of these processes results in the swelling of the subapical part of the stolon. During enlargement, tubers become the largest nutrient sink of the potato plant storing large amounts of carbohydrates (mainly starch) and also significant amounts of protein. Furthermore, potato tubers decrease their general metabolic activity and as such behave as typical storage sinks. Potato tubers of varieties such as Ranger Russet, Russet Burbank, Atlantic, G, and H are harvested from 90 to

160 days after planting, which may vary with production area and marketing conditions. Typical potatoes are about 20-24% dry matter, of which approximately 60-80% consists of starch. After potato vines die back, the tuber skins thicken and harden which provides greater protection to tubers during harvest and blocks entry of pathogens. Although dormancy is defined as the absence of visible growth, dormant tuber meristems are metabolically active. In general, rates of many cellular processes such as respiration, transcription, and translation are suppressed during dormancy and non-dividing, dormant tuber meristems are arrested in the G-1 phase (a period prior to the synthesis of DNA in mitosis). Activation of cell metabolism during early spring triggers the development of sprouts from the eyes of the primary tuber.

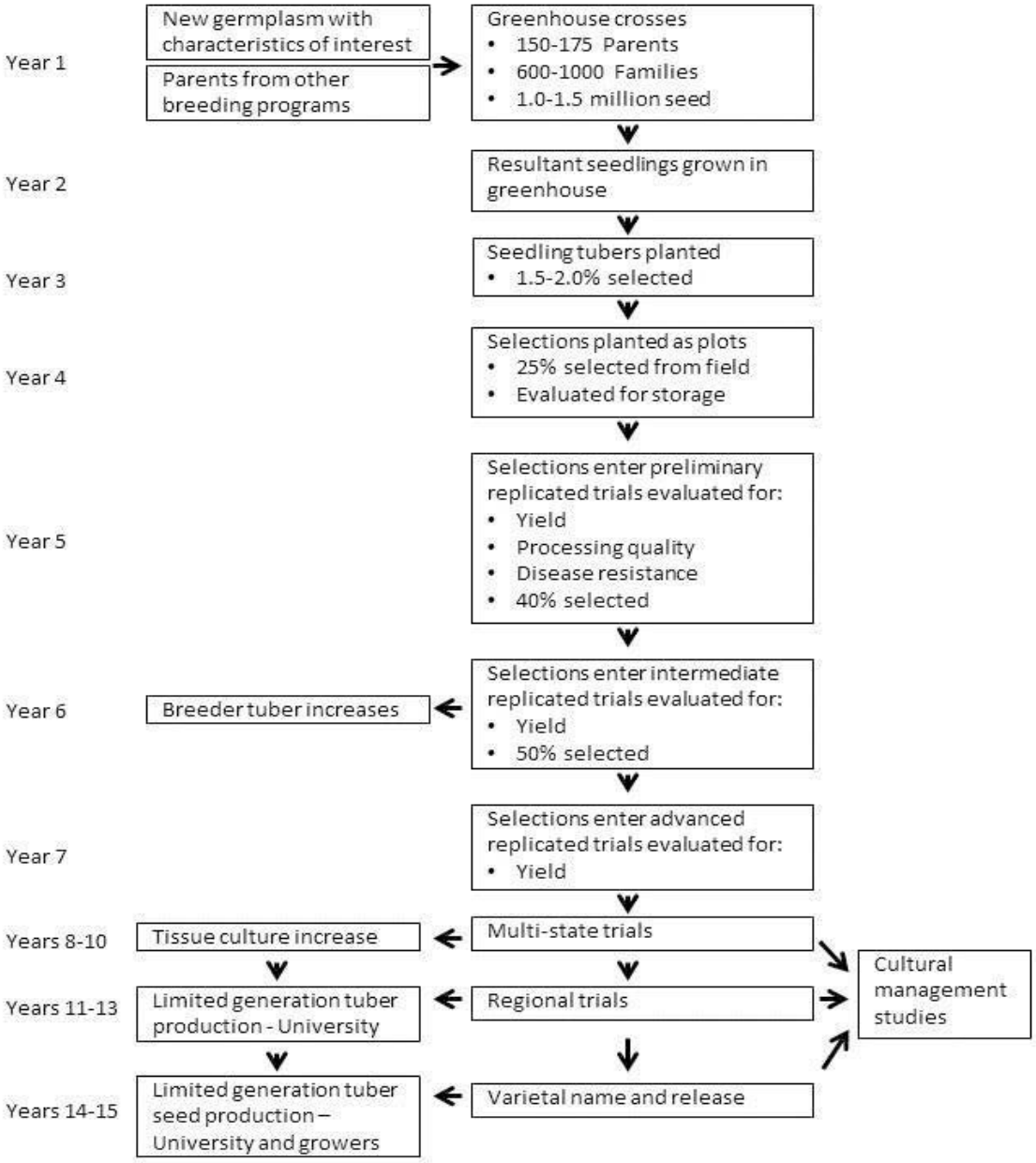
2.6. Seed Propagation

Potato is clonally propagated, which means that tubers rather than seeds are used for planting. A single potato plant produces approximately 12 tubers, therefore it takes multiple years to propagate a new potato variety and generate sufficient amounts of tuber seed needed for commercialization. A typical seed bulk-up program would progress as follows. In the initial year, propagation in tissue culture would produce about 100 plants, and then these plants are grown in a greenhouse to produce thousands of mini-tubers, also called “nuclear seed”. In the second year, mini-tubers are planted at field sites with the lowest possible incidence of diseases and pests, producing generation-1 (G1) seed. Those G1 tubers are grown in the third year to increase the amount of tubers, producing G2 seed. In the fourth, fifth, and sixth years, bulk-up continues with commercial seed often representing G3, G4, and G5 tubers respectively.

2.7 Variety Development

Potato varieties take many years to develop (see **Figure 1**). The decision to establish a new variety is based on many factors such as need in the market place, potential consumer acceptance, and pest tolerance or resistance. Potato varieties do not have a high frequency of introduction and discontinuation compared to some other crops such as field corn or soybeans. Since potatoes are clonally propagated, there is a reduced risk of varietal dilution due to cross pollination.

Figure 1. Potato Variety Development Schematic



2.8. Recipient Potato Varieties

The potato varieties chosen for modification all represent significant value to the potato industry and relatively large percentage of the overall acreage (**Table 2**).

Table 2. Recipient Potato Material

Variety	Usage	Percent of Seed Acreage in US[1]
Russet Burbank	Fries	22.0
Ranger Russet	Fries	6.3
Atlantic	Chips	2.6
G	Chips	2.0
H	Chips	0.7

[1]2009 data for seed potatoes: http://www.nationalpotatocouncil.org/NPC/resources_statistics.cfm

2.9. Typical Agronomic Practices

Examples of typical agronomic practices for a successful potato crop are described in **Table 3**.

Table 3. Example of Agronomic Inputs for Two Potato Variety Classes

	Russet Varieties	Round White Varieties
Planting Date	April 1 to May 10	April 1 to May 10
Planting Rate	15,000 - 18,000 seed pc or 17 – 23 cwt/A	17,000 – 19,000 seed pc or 19 – 24 cwt/A
Row Spacing	34-36” between rows	34-36” between rows
Seed Spacing	10-12” within row	8-10” within row
Fertilizer	For 600 cwt/A yields and optimum soil test levels: 250 lb N – 100 lb P_2O_5 – 330 lb K_2O per acre	For 600 cwt/A yields and optimum soil test levels: 250 lb N – 100 lb P_2O_5 – 330 lb K_2O per acre
Yield/Plant	2-4 lb	2-4 lb
Yield/Acre	400-700 cwt/A	400-700 cwt/A
Harvest Date	September 1 to October 15	August 1 (early season varieties only) to October 15

2.10. Pollination and Outcrossing

Potato is clonally propagated, which means that tubers rather than seeds are used for planting. Harvested tubers are either used for food (~90%), feed (<5%), industrial purposes (<2%) or as “seed” for planting (5%). Therefore pollination to produce seed is not a factor in major commercial potato production, *e.g.*, if Innate™ potatoes were planted in close proximity to organic potatoes, any cross pollination would not impact the organic tubers.

Some of the most important potato varieties including Russet Burbank, produce few flowers and are male sterile. The variety H produces few if any flowers and exhibits male infertility. Many cultivars flower less than wild material, and flowers that do form often drop after pollination resulting in no berry formation. Flowering has no impact on tuber development.

Approximately 80% of seed produced by fertile varieties is derived from self-pollination (Plaisted 1980). Cross-pollination is typically mediated by certain insects including bumblebees (*Bombus* spp.) that

typically don't travel much more than 3 kilometers, rather than wind pollination (OECD 1997). Additionally, other bee species such as honey bees (*Apis mellifera*) are not pollinators of potatoes since potato flowers do not contain ample nectar (OECD 1997). Field evaluations in New Zealand, the United Kingdom and Sweden demonstrated through genetic testing that outcrossing rates were zero when receiving plants were separated by more than 20 meters from genetically modified plants (Conner and Dale 1996). Additionally, potato pollen grains are extremely temperature sensitive. Pallais *et al.* (1988) showed that pollen viability decreased by 30 to 70% when exposed to temperatures of 30°C for up to 30 minutes.

2.11. Wild potatoes in the U.S.

This discussion is based on data in the U.S. Potato Genebank and on a monogram published by the American Society of Plant Taxonomists entitled Wild Potatoes (*Solanum* section *Petota; Solanaceae*) of North and Central America (Spooner *et al.* 2004). The USDA maintains the US Potato Genebank and participates in the Association of Potato Inter-genebank Collaborators (APIC), which has produced a global inventory of wild potato genetic resources available on the internet, the Inter-genebank Potato Database (IPD 2011), which includes the U.S. Potato Genebank records. Many of the IPD records from the U.S. were obtained by John Bamberg and associates (including Spooner) during a decade of collecting wild potatoes in the Southwest U.S. (Bamberg *et al.* 2003).

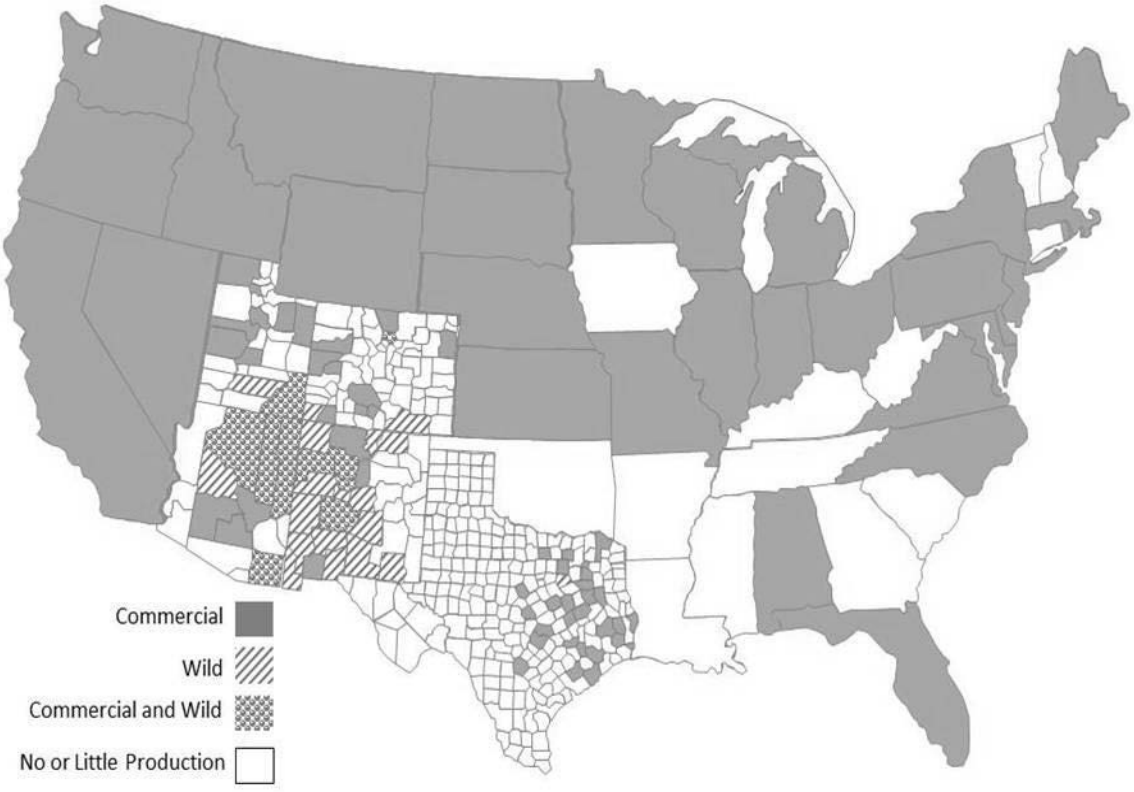
The only two wild potato species that grow within the borders of the USA, and for which specimens exist in gene banks, include the tetraploid species *S. fendleri* (recently reclassified as *S. stoloniferum*; however, some sources, including the IPD, still use the *S. fendleri* designation) and the diploid species *S. jamesii* (Bamberg *et al.* 2003; IPD 2011; Bamberg and del Rio 2011a; Bamberg and del Rio 2011b; Spooner *et al.* 2004). Love (1994) reported that a third species, *S. pinnatisectum*, is also a native species in the USA. However, Spooner *et al.* (2004) determined that what was previously thought to be *S. pinnatisectum* was in fact *S. jamesii.* Through more than 10 years of field work and assessments of existing records, Bamberg *et al.* (2003) and Spooner *et al.* (2004) established the presence of only these two species, *S. fendleri* and *S. jamesii,* in the U.S. These researchers also attempted to verify previously recorded locations, and through this process, updated the maps of current known locations of these species, providing latitude and longitude locations for each documented population (Bamberg *et al.* 2003) and distribution maps (Spooner *et al.* 2004). These species mostly reside in dry forests, scrub desert, and sandy areas at altitudes of 5,000 to 10,000 feet, well isolated from most commercial production areas (Bamberg and del Rio 2011a).

While there is some overlap between the acreage used for commercial production and occurrence of wild species on a county level, the majority of the potato production in the United States is not in wild potato zones (**Figure 2**). However, there is a possibility that a few wild potato plants may be growing near potato fields (Love 1994). Spooner *et al.* (2004) describe *S. jamesii* habitat in the U.S. as among boulders on hillsides, sandy alluvial stream bottoms, in gravel along trails or roadways, rich organic soil of alluvial valleys, sandy fallow fields, grasslands, juniper-pinyon scrub deserts, oak thicket, coniferous and deciduous forests at elevations between 4,500 to 9,400 feet. They describe *S. fendleri* habitat similarly, and at elevations between 4700 to 11,200 feet. The risk of hybridization between commercial varieties and wild species is low because of the lack of geographical overlap and the fact that potatoes are grown from tuber seed. Furthermore, studies discussed in Conner and Dale (1996) indicate that a separation of 20 meters will be sufficient to prevent outcrossing between wild and commercial potatoes.

Love (1994) evaluated the risk associated with growing transgenic potatoes in Canada and the USA and concluded that, given the number and potency of barriers to hybridization and more specifically to introgression and stabilization, the only sound conclusion is that gene introgression into wild *Solanum* species will not occur under natural conditions in these geographies. Therefore, according to Love (1994), potato gene movement from commercial fields to wild potato species would not occur.

Figure 2. Potato Production in the United States

(County Information is for states with wild potato populations (Hijmans and Spooner 2001)



Based on conversations with Bamberg, Love (1994) reported that no one has ever reported finding hybrids between native and cultivated potatoes in the U.S., although gene transfer has been accomplished using special laboratory techniques (Love 1994). Love concluded that, based on the barriers that exist (including geographic isolation as described previously), endosperm imbalances, and multiple ploidy levels, natural hybridization is highly unlikely, and gene introgression (cross hybridization over multiple generations) is impossible or at least highly improbable (Love 1994). More recently, the US EPA has concluded that, based on its review of the scientific literature, successful gene introgression between native and cultivated potatoes in the U.S. is virtually excluded due to constraints of geographical isolation and other barriers to natural hybridization. These barriers include incompatible (unequal) endosperm balance numbers that lead to endosperm failure and embryo abortion, multiple ploidy levels and incompatible mechanisms that do not express reciprocal genes to allow fertilization to proceed. No natural hybrids have been observed between these species and cultivated potatoes in the U.S. (US EPA 2011). Based upon these biological barriers to hybridization, it is unlikely that inter-species pollination would occur with the Innate™ potatoes that are the subject of this petition.

2.12. Weediness

Standard growing practices for potatoes make it highly unlikely that potatoes would persist in a field from one crop cycle to the next. Where potatoes are a rotation crop, as is often the case, other crops such as alfalfa, corn or wheat would be grown following potatoes. In that situation, any potatoes left in the field would be eliminated by tilling, field preparations with herbicides, and harsh winters. In the rare event that potatoes survived mixed in with another crop, they have to face the same challenges during the next year.

Potatoes are typically grown on a 3 year minimum rotation to minimize soil-borne disease buildup such as white mold, pink rot, *Pythium* leak, *Verticillium* wilt, and powdery scab (Hopkins *et al.* 2003). Potatoes left to grow in the field are not desired as they can harbor disease, have no protection with fungicide, and could compete with the desired rotation crop the following year. For these reasons, it is standard practice for potato growers to monitor and control such growth, either with herbicide or tillage. Soil is disturbed greatly during potato growth and harvest operations, therefore requiring tillage prior to planting the rotation crop. Typically a broad spectrum pre-emerge herbicide is applied in the rotation crop to control a wide variety of weeds. Potato growers rarely leave the ground fallow following potato to maximize economic return. If potatoes grew inadvertently in the field left fallow, they would not be protected with insecticide or fungicide and would be susceptible to insects such as Colorado potato beetle and diseases such as early blight, further decreasing the chance of survival and reproduction.

Outside of cultivation, potato seedlings from tubers have difficulty establishing themselves as they cannot compete effectively with other plants (Love 1994). Potato is not known as a colonizer of unmanaged ecosystems. In climax vegetation, potatoes are not able to compete with other species such as grasses, trees and shrubs (CFIA 1996), virtually eliminating any chance for them to become feral. Furthermore, potatoes generally do not cause toxic effects to other plants or animals (including non-target organisms) making pest potential negligible.

Although almost all growers use tubers for planting, there is a small market for “true potato seed” (TPS). TPS is seed that is produced as a result of pollination. The seed is produced inside fruits that resemble small tomatoes on the potato vine. The major disadvantage of TPS is that it segregates for numerous traits because potato is highly heterozygous and each seed produced is potentially a new variety. When TPS is used for planting, harvested tubers will be variable in quality. TPS plants take longer to establish themselves and set tubers, resulting in lower yield compared to plants grown from tuber seed. Some growers in tropical areas use TPS because it is easy to ship and free of pathogens (Dwelle 2003). Love (1994) reported that botanical seed (TPS, not tubers or “seed potatoes”) can survive and germinate for periods of time in excess of seven years however, it is unlikely that plants from TPS would persist for the reasons stated above.

2.13. Conclusions: The Biology of Potato

The propagation of commercial potato varieties through cloning mitigates concerns about increased pest potential such as seed dispersal, survival outside of cultivation, or outcrossing. Potatoes are rarely grown from true potato seed because the offspring would show such extreme diversity they would not be useful as commercial potatoes. In addition, potatoes are not known to escape from commercial fields or show weediness potential. Wild potato varieties are rare in the United States and

geographically isolated from commercial production areas, further reducing concerns about cross-pollination with wild species.

An understanding of the biology of potatoes shows the extremely difficult challenges associated with simultaneously incorporating multiple traits through traditional breeding. Thus, potatoes benefit greatly by the use of Innate™ technologies which provide a superior alternative to breeding, through the addition of non-coding DNA, and result in new potato varieties that are not plant pests.

3. Method of Development: Description of Marker-Free DNA Transformation

3.1. Introduction

Simplot's Innate™ technologies comprise many aspects of plant biology all working together to produce traits of interest in transformed plants. Specifically, the potatoes were modified to reduce asparagine and black spot bruise. Along with the potato derived expression cassettes, are promoters and spacers, also from the potato genome, all creating the gene silencing system. The targeted genes are then combined with the backbone elements of pSIM1278, to create the vector that is inserted into *Agrobacterium.* Potato plants are transformed by *Agrobacterium,* resulting in the incorporation of the expression cassettes that result in the desired traits of interest. A part of this process is the selection of potatoes to receive the new technology, and in this case, we chose some of the most prominent varieties for processing chips and fries. The pSIM1278 acts by effectively reducing asparagine which leads to reduced acrylamide levels in cooked potatoes, as well as reducing black spot bruise incidence.

Description of the Transformation System
The C58-derived *Agrobacterium* strain AGL1 was developed by precisely deleting the transfer DNA of the hyper-virulent plasmid pTiBo542 (Lazo *et al.* 1991). Transformed plants were grown on media containing the antibiotic timentin, which prevents survival of *Agrobacterium,* thus selecting for plants that are free of *Agrobacterium.* At the conclusion of the selection process, all *Agrobacterium* has been removed, no antibiotics remain, and the result is that genomic DNA from the potato or sexually compatible species have been added to the new plants.

Transformation Method
Stock plants were maintained in magenta boxes with 40 ml half-strength M516 medium containing 3% sucrose and 2 g/l gelrite (propagation medium). Potato internode segments of four to six mm were cut from four-week old plants, infected with the *Agrobacterium* AGL1 strain carrying pSIM1278, and transferred to tissue culture media containing 3% sucrose and 6 g/l agar (co-cultivation medium). Infected explants were transferred, after two days, to M404 medium containing 3% sucrose, 6 g/l agar and 150 mg/l timentin to eliminate *Agrobacterium* (hormone-free medium). Details of the methods are described in Richael *et al.* (2008).

Although *Agrobacterium* is effective in cleaving at the Right Border (RB) site, it often failed to fully release the DNA insert from its plasmid vector by also cutting at the Left Border (LB) site (Gelvin 2003). Consequently, some infected plant cells received the DNA insert itself as well as additional vector backbone sequences containing the backbone marker gene for cytokinin. These cells started expressing the isopentenyltransferase (*ipt*) gene resulting in production of the plant hormone cytokinin, which commonly regulates growth and development processes in plants.

After one month, the infected explants were transferred to fresh medium lacking any synthetic hormones and incubated in a Percival growth chamber under a 16 hr photoperiod at 24^{o} C where they started to form shoots. Many shoots expressed the *ipt* gene and displayed a cytokinin overproduction phenotype; these shoots were discarded and not considered for further analyses. PCR genotyping demonstrated that about 0.3 to 1.5% of the remaining shoots contained at least part of the DNA insert while lacking the *ipt* gene. Details on *ipt*-based marker-free plant transformation were published by Richael *et al.* (2008).

The process of eliminating *Agrobacterium* started two days after explant infection. For this purpose, tissues were subjected to the antibiotic timentin (150 mg/l) until proven to be free of live *Agrobacterium*. Proof was obtained by incubating stem fragments of transformed events on nutrient broth-yeast extract (NBY medium) for 2 weeks at 28^{O}C (repeated twice). In accordance with 7 CFR Part 340, transformed plants were transported and planted in the field only when free of live *Agrobacterium*. The potato events were confirmed to be free of *Agrobacterium*-derived vector backbone DNA by the following three methods: 1) If plants had the negative selectable isopentenyl isomerase (*ipt*) marker gene in the vector backbone, they were discarded. Plants with *ipt* gene expression would have stunted phenotypes, abnormal leaves, or the inability to root due to the cytokinin overproduction caused by *ipt* expression; 2) Absence of the backbone DNA was confirmed with Southern blot hybridization; 3) PCR was used to confirm no fragments of the backbone DNA were present.

3.2. Parental Varieties

Russet Burbank. Luther Burbank developed this variety in the early 1870s. Plants are vigorous and continue vine growth throughout the season. Stems are thick, prominently angled and finely mottled. Leaflets are long to medium in width and light to medium green in color. The blossoms are few, white and not fertile. The cultivar is tolerant to common scab but is susceptible to *Fusarium* and *Verticillium* wilts, leafroll and net necrosis and virus Y. Plants require conditions of high and uniform soil moisture and controlled nitrogen fertility to produce tubers free from knobs, pointed ends and dumbbells. Jelly-end and sugar-end develop in tubers when plants are subjected to stress. The tubers produced are large brown-skinned and white-fleshed, display good long-term storage characteristics, and represent the standard for excellent baking and processing quality. The variety is sterile and widely grown in the Northwest and Midwest, especially for the production of french fries.

Ranger Russet. This full season variety was released in 1991. Ranger Russet is more resistant than Russet Burbank to *Verticillium* wilt, viruses X and Y, leafroll and net necrosis, and *Fusarium* dry rot. It is highly resistant to hollow heart. Plants are large and upright to spreading. Stems are thick, green that can be light brownish to light purple in full sun. Leaves are large, broad and medium green. Flowers are abundant and produce viable pollen. Buds are green with reddish-purple base and pedicel and moderate amount of short pubescence. Corolla is medium large, red-purple color and anthers are bright yellow. It produces high yields of good quality, high specific gravity tubers that are long and slightly flattened, and well suited for baking and processing into french fries. Tubers are susceptible to common scab and black spot bruise. Ranger Russet matures earlier than Russet Burbank and would be considered a medium-length storage variety. The variety is fertile and mainly grown in the Northwest, especially for the production of french fries.

Atlantic. Plants are moderately large, with thick, upright stems, and slightly swollen, sparsely pubescent nodes. Leaves are bright, medium green, smooth, and moderately pubescent with prominent wings, large asymmetrical primary leaflets and numerous secondary and tertiary leaflets. Flowers are profuse

with green, awl-shaped, pubescent calyx lobes, pale lavender corolla, orange anthers and abundant, viable pollen. The cultivar is tolerant to scab and *Verticillium* wilt, resistant to pinkeye, highly resistant to Race A of golden nematode, virus X, tuber net necrosis, and shows some resistance to black spot bruise. Tubers are susceptible to internal heat necrosis, particularly in sandy soils in warm, dry seasons. Hollow heart in the larger diameter tubers (diameter > 4 inches) can be serious in some growing areas. Tubers are oval to round with light to heavy scaly netted skin, moderately shallow eyes, and white flesh. Tuber dormancy is medium-long. With high yield potential, high specific gravity and uniform tuber size and shape, Atlantic is the standard variety for chipping from the field or from very short-term storage (Webb *et al.* 1978). The variety is fertile and mainly grown in the Northeast and Southeast, especially for the production of chips.

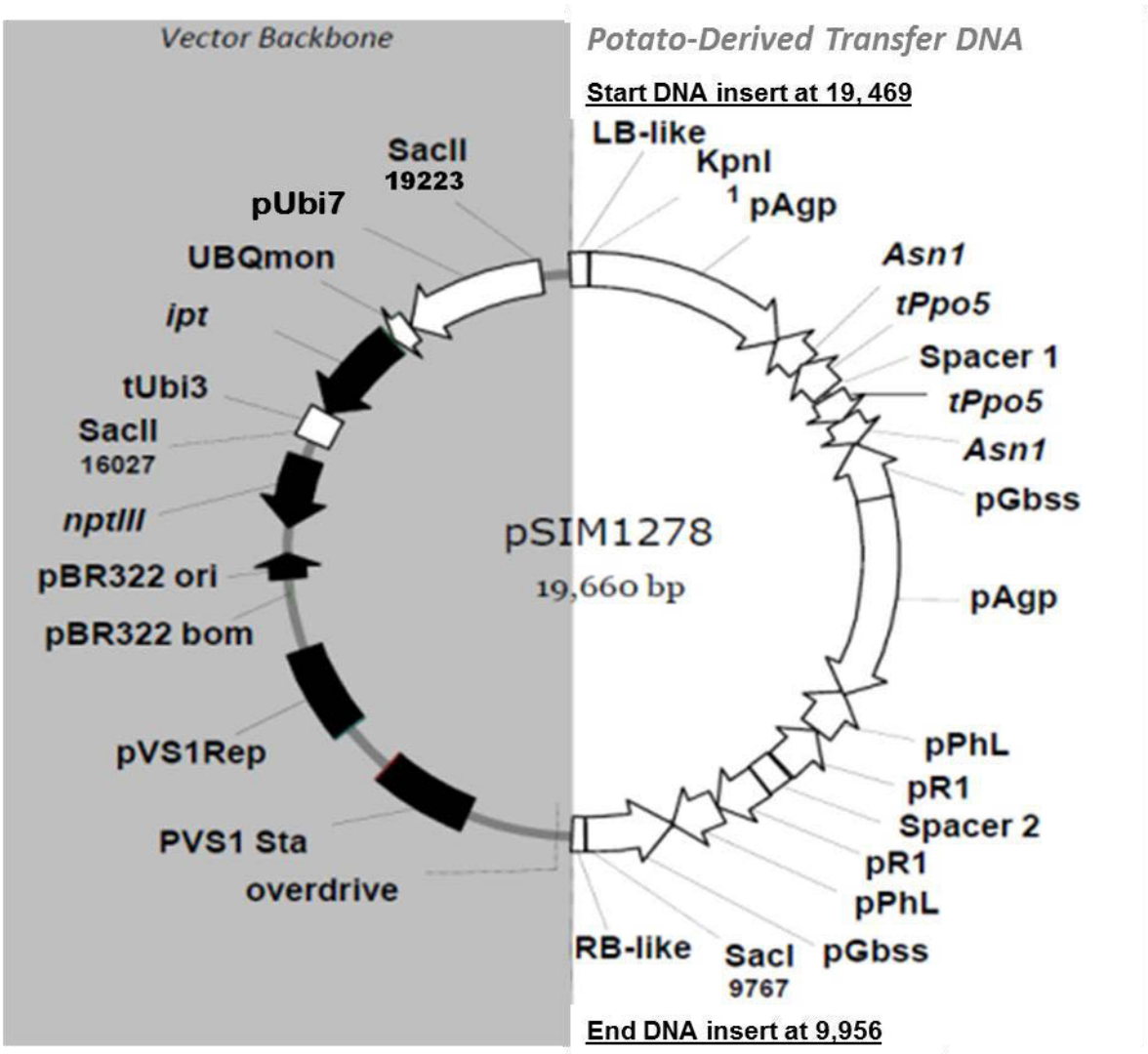
Variety G. Mid-season chipping variety with medium size tubers, round shape, shallow eyes, smooth slightly netted skin, white flesh, and high solids. It can be used as a fresh crop variety through mid-term storage. It generally has low defects but can display pressure bruise late in the storage season. It appears to be fairly resistant to PVY, is tolerant to common scab, and is confirmed to have resistance to the golden nematode *Globadera rostochiensis* Ro1. It has moderate flowering with white flowers which are both male and female fertile.

Variety H. Mid to late season chipping variety with large round to oval tubers, somewhat flattened shape, slightly indented eyes, smooth tan skin, pale yellow flesh, and has low to medium solids relative to other chipping varieties. It is generally used as a mid to late season storage variety. It is resistant to tuber early blight but susceptible to common scab. It has sparse white flowers and has no male fertility and very low female fertility.

3.3. Plasmid used for Transformation

The plasmid pSIM1278 is a binary transformation vector consisting of two parts: vector backbone and the DNA insert (**Figure 3**). The backbone section contains well-characterized bacterial origins of replication from plasmids pVS1 and pBR322, and the *nptIII* gene for bacterial resistance to kanamycin (see **Table 4** for details of the backbone of pSIM1278). An expression cassette comprising the *Agrobacterium ipt* gene preceded by the polyubiquitin (Ubi7) promoter (GenBank accession no. U26831.1) and followed by the Ubi3 terminator was introduced as a 2.6-kb SacII fragment into the vector backbone (Garbarino and Belknap 1994). The DNA insert consists of the genetic elements described in **Table 5**.

Figure 3. Vector pSIM1278



The vector backbone region, on the left, starts at position 9,957-bp and ends at 19,468-bp (= 9,512-bp). The backbone DNA consists mainly of bacterial DNA and is intended only to support maintenance of the DNA insert prior to plant transformation. The DNA insert region, including flanking Border sequences, is on the right from 19,469-bp to 19,660 and from 1-bp to 9,956, which is a total of 10,148-bp.

3.4. Backbone Portion of pSIM1278

The purpose of the backbone portion of pSIM1278 is to maintain the DNA insert in bacteria; it is not intended for transfer to plant cells. The various elements of the backbone are described in **Table 4**.

Table 4. Genetic elements of the pSIM1278 backbone

Genetic Element	Origin	Intended Function	Other effects on plant	Genbank Accession Number	Start-End Point in pSIM1278 (bp)	Reference
SacII restriction site	*S. tuberosum*	Site for connection of Ubi7 promoter with LB flanking sequence.	*None*	AJ272136.1	19,219-19,224	
Polyubiquitin promoter (**Ubi7**) including the coding sequence for a 76-amino-acid potato ubiquitin monomer (**UBQmon**)	*S. tuberosum* var. Ranger Russet	Drives expression of the *ipt* backbone marker gene	*None*	U26831.1	17,479-19,218	
Isopentenyl transferase (***ipt***) gene	*Agrobacterium tumefaciens*	condensation of AMP and isopentenylpyrophosphate to form isopentenyl-AMP, a cytokinin	*Cytokinin formation*	NC_002377.1	16,744-17,466	Smigocki and Owens 1988
Terminator of the ubiquitin-3 gene (**tUbi3**)	*S. tuberosum*	Terminate *ipt* gene transcription	*None*	GP755544.1	16,038-16,392	Garbarino and Belknap 1994
Neomycin phosphotransferase III (***nptIII***) gene	*E. coli*	Aminoglycoside phosphotransferase	*None*	FJ362602.1	15,048-15,842	Courvalin *et al.* 1977
Origin of replication for pBR322 (**pBR322 ori**)	*E. coli*	Start position for plasmid replication in bacterial cells	*None*	J01784.1	14,477-14,757	*
(**pBR322 bom**)	*E. coli*	pBR322 region for replication in *E. coli*	*None*	J01749.1	14,077-14,337	*
pVS1 replicon (**pVS1Rep**)	*Pseudomonas fluorescens* plasmid pVS1	pVS1 region for replication in *Agrobacterium*	*None*	AJ537514.1 (4,501-5,501)	12,667-13,667	*
pVS1 partitioning protein StaA (**PVS1 Sta**)	*Pseudomonas fluorescens* plasmid pVS1	pVS1 stability	*None*	AJ537514.1 (6,095-7,095)	11,074-12,074	*
overdrive	*Agrobacterium tumefaciens*	Enhances cleavage at the Right Border site	*None*	K00549.1 (103-132)	9,963-9,992	*

*: see: http://www.cambia.org/daisy/cambia/585.html - (General structure map of pCAMBIA vectors)

3.5. The Transfer DNA (DNA insert) of the Vector

The transfer DNA (DNA insert) consists of non-coding genetic elements described in **Table 5**. The DNA insert or a functional part thereof, is the only part of vector pSIM1278 that is present in selected transformed events.

Table 5. Genetic elements of the DNA insert of pSIM1278, from Left Border site to Right Border site

Genetic Element	*Origin*	Intended Function	Genbank Accession Number	Start-End Point in pSIM1278	Reference
1. Left Border (LB) site[1]	Synthetic	Site for secondary cleavage to release single-stranded DNA insert from pSIM1278	AY566555[2] (bases 1-25)	19,469 – 19,493	van Haaren et al. 1989
2. DNA flanking the LB sequence	*S. tuberosum* var. Ranger Russet	Supports secondary cleavage at LB	AY566555[2] (bases 26-187)	19,494 – 19,655	
3. KpnI restriction site	*S. tuberosum*	Site for connection of DNA insert with LB flanking sequence.	AF393847.1	19,656 –1	
4. Promoter for the ADP glucose pyrophosphorylase gene (pAgp), 1st copy	*S. tuberosum* var. Ranger Russet	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of Asn1 and Ppo5, especially in tubers	HM363752	2-2,261	
5. Fragment of the asparagine synthetase-1 (Asn1) gene (1st copy antisense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (9) double stranded RNA that triggers the degradation of Asn1 transcripts to impair asparagine formation	HM363759	2,262-2,666	Chawla et al. 2012[3]
6. 3'-untranslated sequence of the polyphenol oxidase-5 gene (Ppo5) (1st copy, in antisense orientation)	*S. verrucosum*	Generates with (8) double stranded RNA that triggers the degradation of Ppo5 transcripts to block black spot bruise development	HM363754	2,667-2,810	
7. Spacer-1	*S. tuberosum* var. Ranger Russet	Sequence between the 1st inverted repeats	HM363753	2,817-2,973	
8. 3'-untranslated sequence of the polyphenol oxidase-5 gene (Ppo5) (2nd copy, in sense orientation)	*S. verrucosum*	Generates (6) double stranded RNA that triggers the degradation of Ppo5 transcripts to block black spot bruise development	HM363754	2,974-3,117	
9. Fragment of the asparagine synthetase-1 (Asn1) gene (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (5) double stranded RNA that triggers the degradation of Asn1 transcripts to impair asparagine formation	HM363759	3,118-3,523	Chawla et al. 2012[3]
10. 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	One of the two convergent promoters that drives expression of an inverted repeat containing fragments of Asn1 and Ppo5, especially in tubers	HM363755	3,530-4,215	
11. pAgp, 2nd copy	*S. tuberosum* var. Ranger Russet	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	HM363752	4,232-6,491	
12. Fragment of promoter for the potato phosphorylase-L (pPhL) gene (1st copy, in antisense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (16) double stranded RNA that triggers the degradation of PhL transcripts to limit the formation of reducing sugars through starch degradation	HM363758	6,492-7,000	
13. Fragment of promoter for the potato R1 gene (pR1) (1st copy, in antisense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (15) double stranded RNA that triggers the degradation of R1 transcripts to limit the formation of reducing sugars through starch degradation	HM363757	7,001-7,532	
14. Spacer-2	*S. tuberosum* var. Ranger Russet	Sequence between the 2nd inverted repeat	HM363756	7,539-7,796	

Table 5 continued. Genetic elements of the DNA insert of pSIM1278, from Left Border site to Right Border site

Genetic Element	Origin	Intended Function	Genbank Accession Number	Start-End Point in pSIM1278	Reference
15. Fragment of promoter for the potato R1 gene (pR1) (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (13) double stranded RNA that triggers the degradation of R1 transcripts to limit the formation of reducing sugars through starch degradation	HM363757	7,797-8,328	
16. Fragment of promoter for the potato phosphorylase-L (pPhL) gene (2nd copy, in sense orientation)	*S. tuberosum* var. Ranger Russet	Generates with (12) double stranded RNA containing this sequence will trigger the degradation of PhL transcript to limit the formation of reducing sugars through starch degradation	HM363758	8,329-8,837	
17. pGbss (2nd copy, convergent orientation relative to the 2nd copy of pAgp)	*S. tuberosum* var. Ranger Russet	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	HM363755	8,838-9,761	
18. SacI restriction site	*S. tuberosum*	Site for connection of DNA insert with RB flanking sequence.	AF143202	9,76 – 9,767	
19. DNA flanking the RB sequence	*S. tuberosum* var. Ranger Russet	Supports primary cleavage at RB-Like site	AY566555[2] (bases 231-391)	9,77 – 9,931	
20. Right Border (RB) sequence[1]	Synthetic	Site for primary cleavage to release single stranded DNA insert from pSIM1278	AY566555[2] (bases 392-416)	9,932 - 9,956	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]GenBank Accession AY566555 was revised to clarify the sources of DNA for the Border regions.
[3]ASN1 described as genetic elements 5 and 9 is referred to as StAst1 in Chawla *et al.* 2012.

3.6. Open Reading Frames Associated with the DNA Insert

Open reading frames (ORFs) are long sequences of DNA (a few hundred base pairs) that start with an ATG triplet and end with a stop codon. ORFs may encode part or all of a protein if flanked by appropriate regulatory sequences.

The full 10,148-bp DNA insert, from the cleavage site in the Left Border element to the cleavage site in the Right Border element contains fragments associated with an ORF of the *Asn1* gene and the trailer associated with an ORF of the *Ppo5* gene. These inverted repeats produce a pool of variably-sized RNAs that trigger gene silencing and are not translated into protein when transcribed.

3.7. Conclusions of Methods of Development: Description of Marker-Free DNA Transformation

A description of Innate™ technologies outlines the plant biological systems all working together to create the plants. These include trait identification, design of vectors, incorporation of vectors into *Agrobacterium*, recipient potato variety selection, transforming plants, and confirmation that the new potatoes contain the expected DNA inserts. The Innate™ methods allowed us to insert the desired traits into potato varieties using non-coding DNA into potato and develop new potato events that are not plant pests.

4. Characteristics of Transferred DNA and Gene Regulation

The potato events all produce potatoes that are silenced for specific genes. The silencing methods are part of Simplot's Innate™ technologies for reducing gene expression. This patented method provides the framework for reducing the expression of genes that ultimately reduce black spot bruise, asparagine, sugars, and acrylamide in potatoes. A brief introduction to gene silencing is given here, describing how similar methods are found in nature. In general, the inserted DNA contains silencing cassettes that, when expressed, generate variably-sized and unprocessed transcripts. These transcripts trigger the degradation of mRNAs that would normally code for an enzyme, like asparagine synthetase. This results in much reduced levels of the targeted "silenced" enzymes.

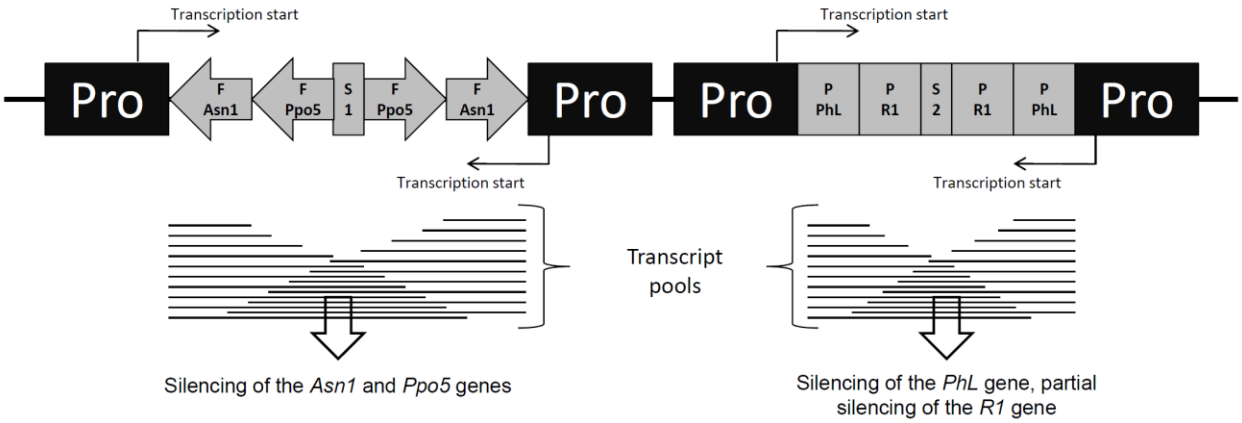
All potato events were analyzed by DNA gel blot analyses to determine the structure and copy number of the integrated DNA insert sequences and to confirm the absence of vector backbone sequences. These studies were carried out as parts of the characterization and biosafety assessment of the events. In addition, molecular characterization was used to determine the sequence of the junctions flanking the DNA insert and show stability of the inserted DNA. Sequencing information of the junctions provided the basis for developing event-specific PCR tests for all events.

4.1. Description and History of the Modification

Mechanism for Gene Silencing

The silencing approach applied here to down-regulate the transcript levels of several genes is illustrated in **Figure 4**. Two copies of a DNA segment comprising fragments of four targeted genes are inserted, as an inverted repeat, between two convergent promoters that are predominantly active in tubers. Plants containing the silencing cassette produce a diverse and unpolyadenylated array of RNA molecules in tubers that dynamically and vigorously down-regulate the intended target genes. The size of the RNA molecules is generally smaller than the distance between the two promoters according to Yan *et al.* 2006.

Figure 4. Silencing Approach Using the DNA Insert of pSIM1278



(Pro = functionally-active promoter, F = gene fragment, S1 = spacer 1, P = non-functional promoter fragment, S2 = spacer 2)

The expression of an inverted repeat is a regulatory system that has evolved naturally as a consequence of the duplication of genes and regulatory elements. One example relates to a 27-kb region of the

soybean genome containing two perfectly repeated and inverted clusters of three chalcone synthase (*Chs*) genes (Tuteja *et al.* 2009). This results in the silencing of all *Chs* gene family members through the formation of double-stranded RNA. In this example, the double-stranded RNA initiates gene silencing, and is an example of naturally occurring inhibition of a metabolic pathway, which functions specifically in one tissue while allowing expression in other parts of the plant.

Expression of Neighboring Genes

Previous studies on convergent transcription have shown that transcripts are of variable size but smaller than the distance between the two promoters, both in yeast (Prescott and Proudfoot 2002) and plants (Yan *et al.* 2006). In a study on convergent transcription-based silencing of the β-glucuronidase (*gus)* gene, no expression of neighboring genes was found (Yan *et al.* 2006). The RNA gel blot analyses presented in this petition (**Appendix 5. Efficacy and Tissue-Specificity of Gene Silencing**) demonstrated the absence of bands or smears indicative of read-through transcripts that hybridized with a probe derived from the entire *Asn1*/*Ppo5* inverted repeat (see, for instance, **Figure 2** of **Appendix 5. Efficacy and Tissue-Specificity of Gene Silencing**).

Although the possibility of rare read-through transcripts cannot be excluded, it is unlikely that such transcripts would interfere with the expression of neighboring genes because single stranded transcripts are ineffective in triggering gene silencing (Yan *et al.* 2006). Furthermore, studies on the expression of an antibiotic selectable marker gene next to a convergent transcription-based silencing construct demonstrated that this antibiotic gene was not affected by expression of the silencing construct (Yan *et al.* 2006). In the unlikely event that read-through occurred, and lowered the expression of an adjacent gene in any of the 10 events, it would have been evident through change in plant phenotype. After extensive evaluations from multiple field trials over three years, no unexpected agronomic, phenotypic, or biochemical differences were observed with these 10 events when compared with control varieties.

4.2. Verification of Insert Integrity and Number of Inserted Sequences

A diagram of the structure of inserts is shown in **Figure 5** (see **Abbreviations** for most keys; AGP = pAgp, GBS = pGbss). Details on characterization of the inserted DNA are provided in **Appendix 1. Characterization of Inserted DNA**. Two events (J78 and G11) contain a single truncated copy of the DNA insert with a functionally-active *Asn1*/*Ppo5* gene silencing cassette (and part of the R1/PhL promoter silencing cassette that is not functionally active).

The comprehensive molecular analyses confirmed anticipated similarities between the events of this petition and T-DNA integration events described elsewhere:

(1) Six events (F10, F37, E12, E24, J78, and G11) contain 1 copy (whereby "copy" implies the presence of at least an *Asn1*/*Ppo5* gene silencing cassette), three events contain 2 copies (J3, J55, and H50), and the remaining event H37 contains 1 copy with 3 additional fragments. This finding indicated that the number of copies of the DNA insert is similar to that reported elsewhere for T-DNAs in potato (Wolters *et al.* 1998) and other crops such as tobacco (De Neve *et al.* 1997; De Buck *et al.* 1999), *Arabidopsis* (De Buck *et al.* 2009), rice (Sallaud *et al.* 2003; Yang *et al.* 2005), wheat (Cheng *et al.* 1997), and barley (Bartlett *et al.* 2008). Although high copy numbers have been linked to post-transcriptional gene silencing (Lechtenberg *et al.* 2003), we found no differences in the extent and persistence of silencing activities between higher-copy events and the events with only one copy.

(2) Event J55 contained two linked DNA inserts positioned as an inverted repeat. Lechtenberg *et al.* (2003) showed with bacterial T-DNA that the presence of a second gene copy either in tandem

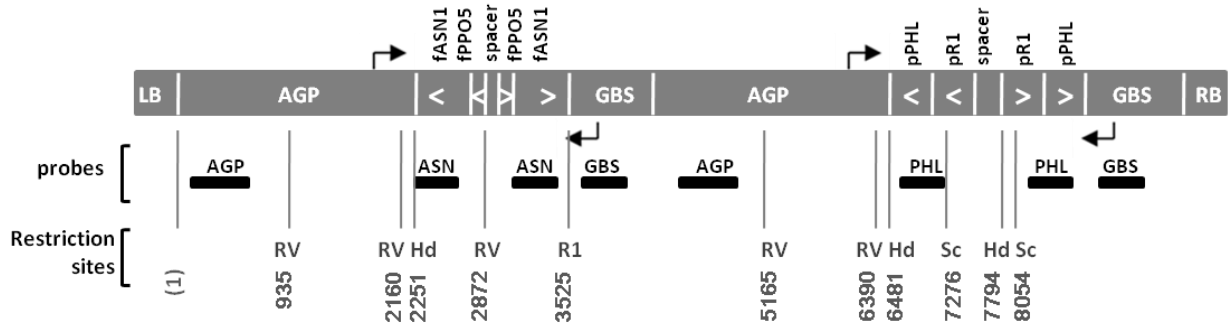
or an inverted arrangement did not result in silencing. Thus, it's likely that the inverted linked DNA insert copies in J55 would not contribute to silencing and therefore, the silencing of targeted genes functions as intended based on the inverted repeats positioned between convergent promoters.

(3) Inserts are occasionally flanked by short DNA sequences that are derived from the plant genome or the DNA insert. These insertions appear to be part of the integration process and occur at rather high frequencies (Windels *et al*. 2003). An example of an event with such sequences includes the 49-bp sequence between the two DNA inserts of J55. A blast search of this short DNA sequence using GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome) partially matched known sequences from *S. tuberosum,* confirming that the origin was most likely from either the plant genome or the DNA insert.

(4) Most transferred DNA inserts are shorter than the full distance between the Left and Right Border sequences, as shown by short deletions near the borders. Such deletions are also associated with T-DNA integration and hypothesized to result from double-strand break repair (Gheysen *et al.* 1991). Short deletions that did not impair the functional activity of any of the two silencing cassettes were found in, for instance, events F10 (a 38-bp deletion at the Right Border) and F37 (a 12-bp deletion at the Left Border). Large deletions at the Right Border side that resulted in loss of the PhL/R1 promoter silencing cassette occurred in events J78 and G11.

Figure 5. Diagram of DNA Inserts

F10 Insert:



F37 Insert:

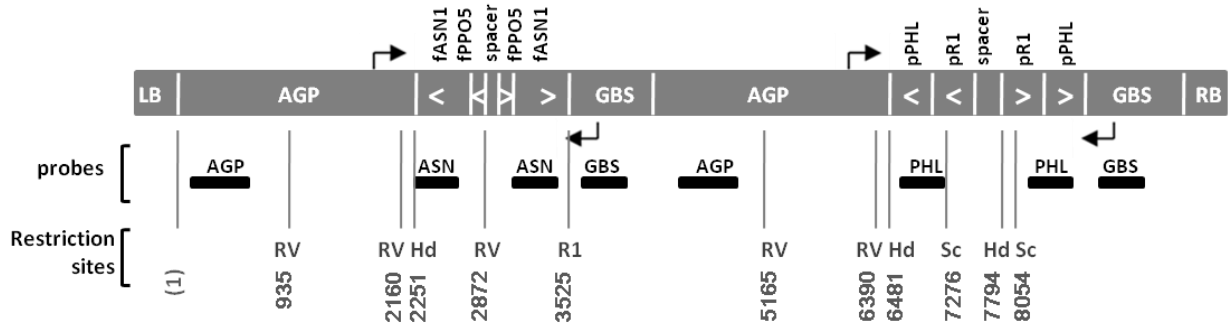
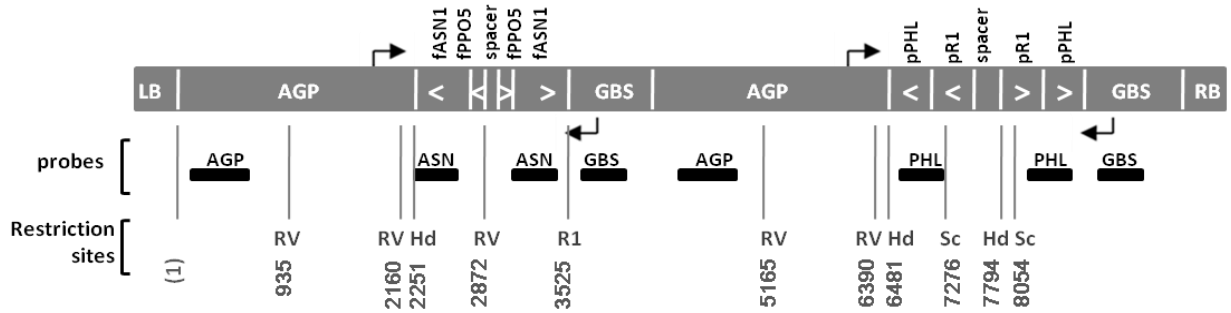
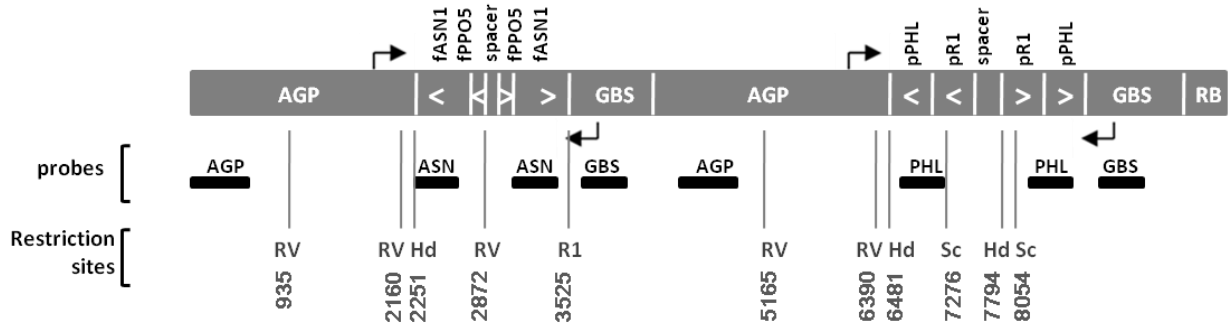


Figure 5 (Continued). Diagram of DNA Inserts

E12 Insert:



E24 Insert:



J3 Insert:

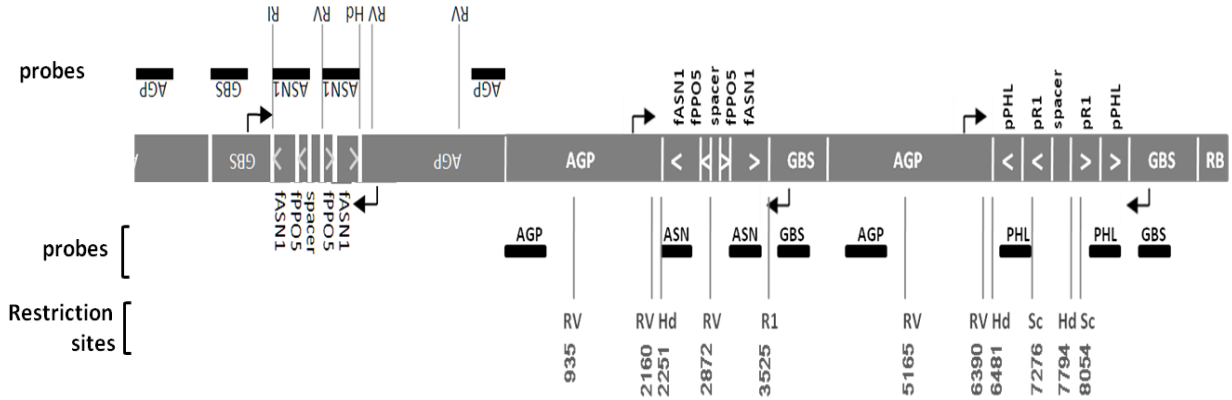
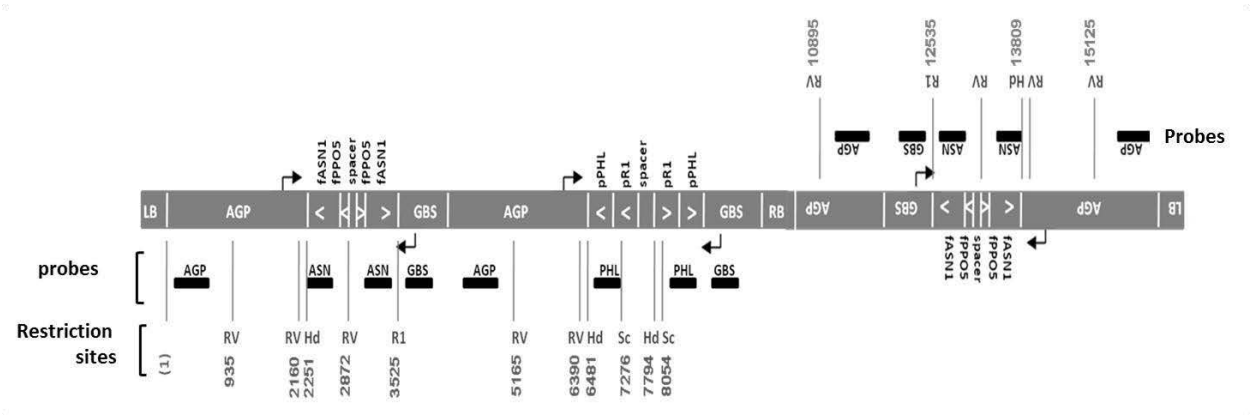
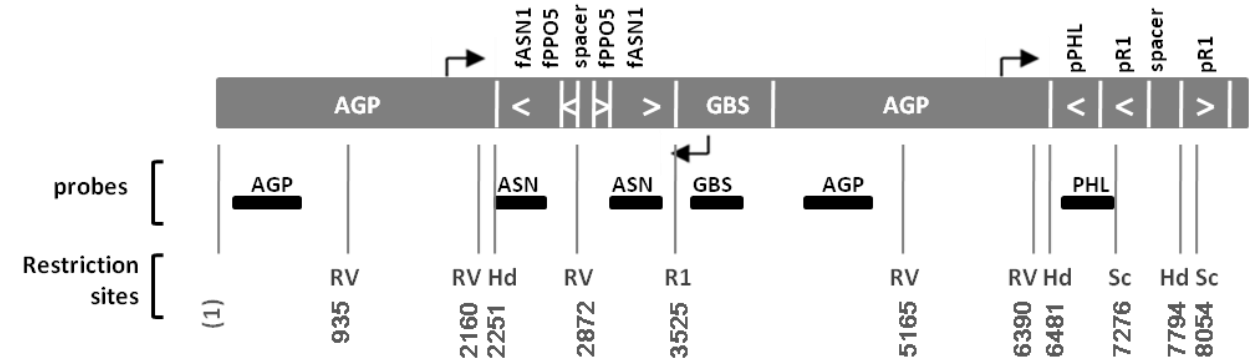


Figure 5 (Continued). Diagram of DNA Inserts

J55 Insert:



J78 Insert:



G11 Insert:

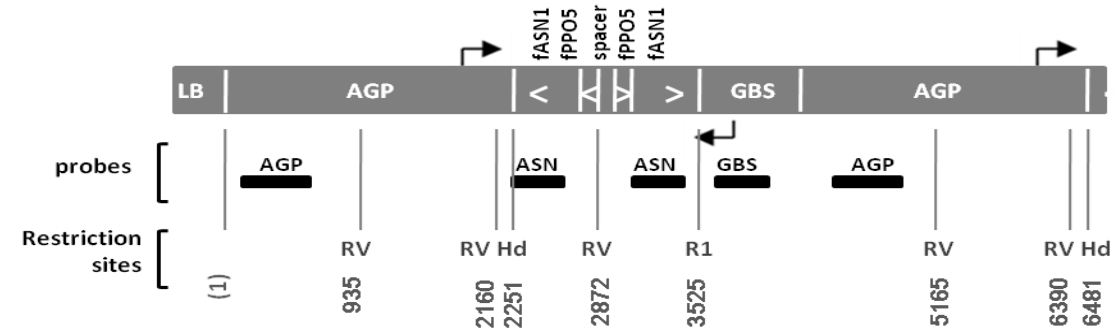


Figure 5 (Continued). Diagram of DNA Inserts

H37 Insert:

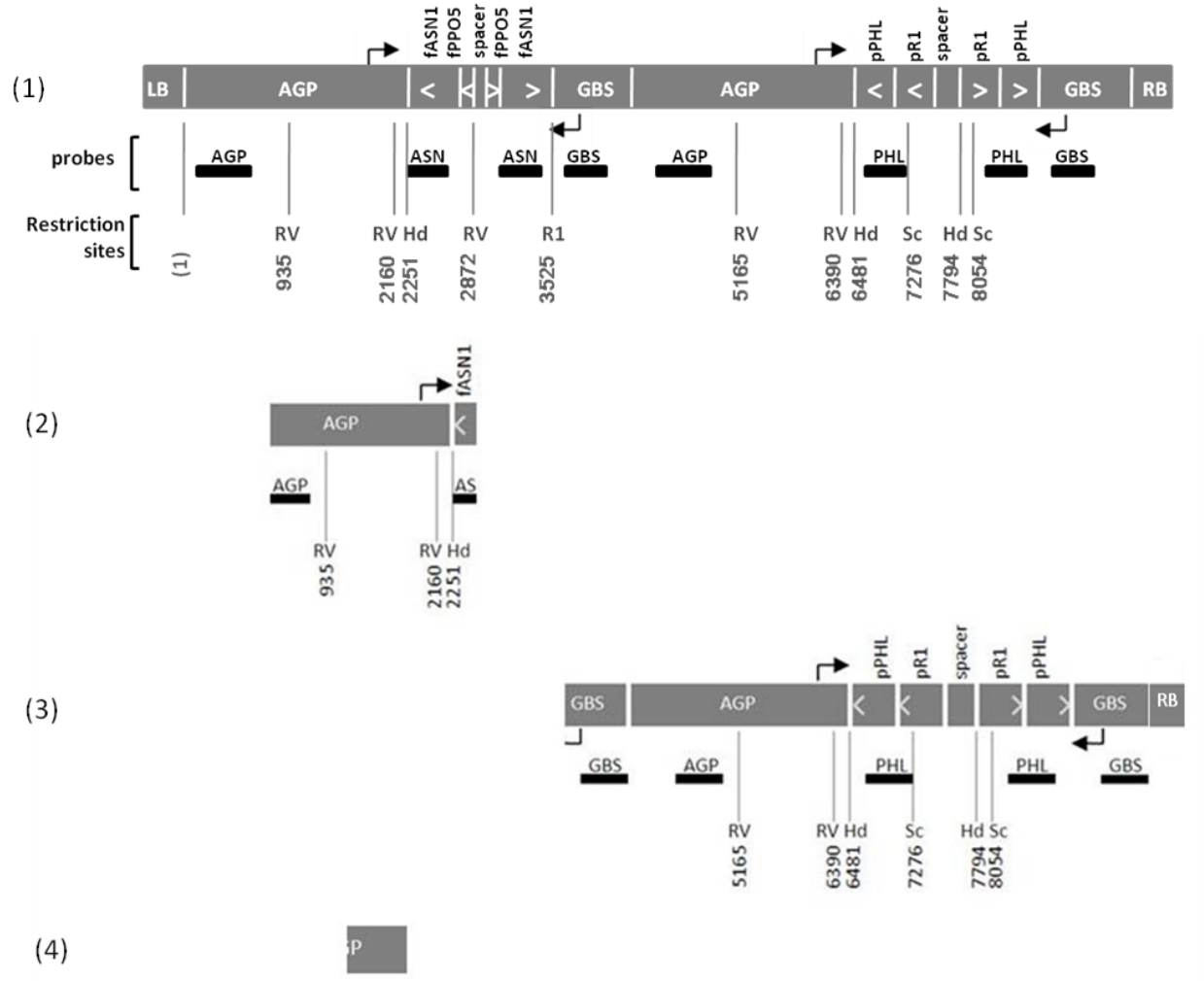
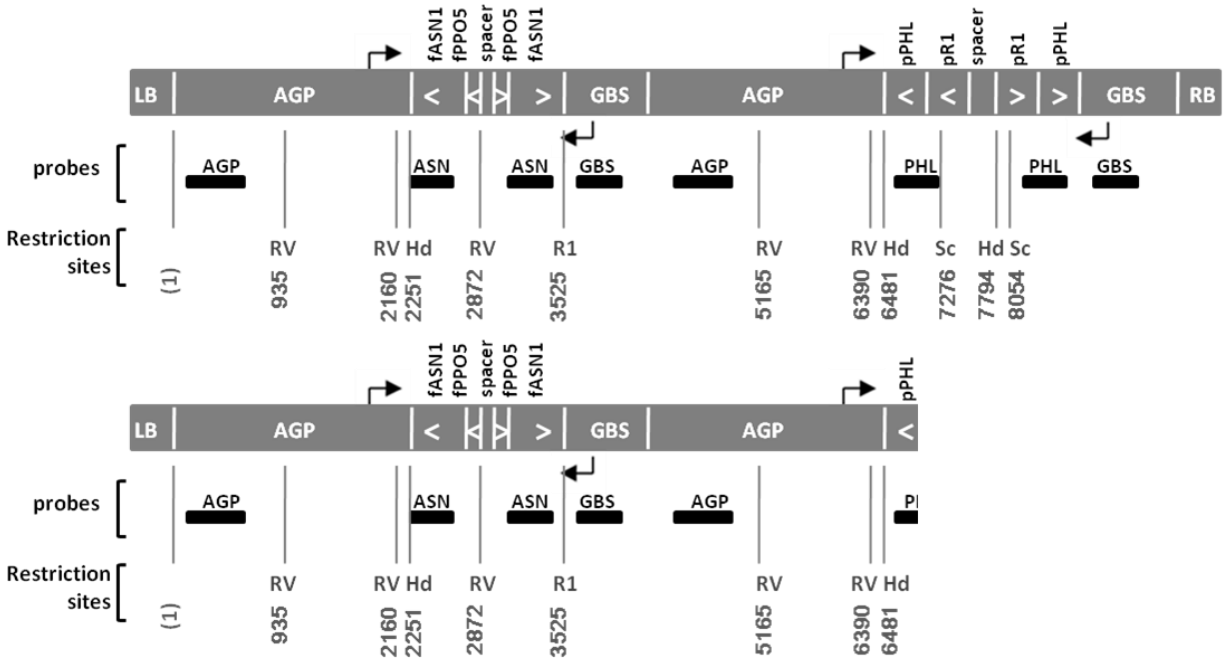


Diagram (4) is a small fragment containing part of the AGP promoter.

Figure 5 (Continued). Diagram of DNA Inserts

H50 Insert:



4.3. Selection of Backbone-free Plants

Agrobacterium-mediated transformation often results in transfer of "backbone" sequences that are vector DNA in addition to the intended sequences positioned between the Left and Right Borders of the plant derived DNA insert. The frequency of transfer of backbone DNA was estimated at 75% for tobacco (Kononov *et al.* 1997) and 47% to 67% for *Arabidopsis* (Oltmanns *et al.* 2010).

In contrast, all the events in this submission were confirmed to be free of vector backbone DNA (**Appendix 2. Evidence for the Absence of Vector Backbone DNA**). This finding confirms previously published work on Simplot's cytokinin vectors (Richael *et al.* 2008).

4.4. Flanking Regions of Inserted Sequences

Extensive research was conducted to characterize the inserted DNA for all events (**Appendix 1. Characterization of Inserted DNA**). In addition, at least one junction was sequenced for all events to develop event-specific PCR methods.

Among the evidence supporting safety relative to the insertion site are the following: (1) most insertions do not disrupt gene expression because DNA integrates at random when no selection is used during transformation (Kim *et al.* 2007) and only approximately 5.5% of the potato genome is occupied by genes (Zhu *et al.* 2008); (2) insertions that do disrupt gene functions are almost always recessive (Hagio *et al.* 2002; Chiou *et al.* 2006; Daxinger *et al.* 2008), which means that change to a single chromosome would be unlikely to result in an observable phenotype (in contrast to most other crops, transformed potatoes are not self-fertilized (backcrossed) to develop events that are homozygous for the insert); and

(3) if an unusual and undesirable phenotype occurred, that event would be selected only if equivalent or superior to the untransformed potato variety.

4.5. Stability of Inserted DNA

Bacterial T-DNAs are not always stable after insertion into a plant. The estimated instability rate of between 0.5-5.9×10^{-4}, is associated with meiosis (Müller *et al.* 1987; Conner *et al.* 1998). Because potato is a vegetatively propagated crop and reproduction does not involve meiosis, DNA insertions were expected to be stable. Nevertheless, the stability of integrated DNA inserts was confirmed both by DNA gel blot hybridization and by assaying G0, G1, G2, and G3 tubers for polyphenol oxidase activity (**Appendix 3. Evidence for Stability of the Inserted DNA**).

It should be mentioned here that one event that is not considered in this submission for nonregulated status, designated as G09, was found to be unstable. About 2% of the G2 tubers of this event had partially lost black spot bruise tolerance (**Appendix 3. Evidence for Stability of the Inserted DNA**). Such instability may have been associated with the unusually complex DNA insert organization of G09 (7 copies of at least part of the DNA insert). While not certain that the large copy number was the cause of instability, it would be recommended to select events with the minimum number of copies needed to express the desired traits. For example, it would be desirable to select future events with relatively simple DNA insertions (2 copies or less).

Although instability is undesired and may result in a phenotypic reversion back to wild-type where tubers lose the incorporated traits (black spot bruise tolerance, reduced acrylamide-forming potential, and, in some cases, reduced formation of fructose and glucose), it would not trigger any biosafety issues. To date, all assessments have been limited to G0, G1, G2, and G3 material. Continued testing will monitor phenotypic stability of the new traits.

4.6. Event-Specific PCR

As part of our stewardship and identity preservation plan as well as for internal event detection, event-specific PCR was developed for all the events submitted for nonregulated status. The methods developed will be used to monitor plants and tubers in field and storage as part of quality management programs (**Appendix 4. Junction Analysis and Event-Specific Detection**).

4.7. Conclusions: Characteristics of transferred DNA and Gene Regulation

We found the 10 events to contain at least 1 full or partial copy of the DNA insert from pSIM1278 and, in the case of H37, three additional fragments of pSIM1278. Although the inserts were not always full-length, therefore, each event contained at least one functional *Asn1*/*Ppo5* gene silencing cassette. This means there are three main differences between these events and their untransformed counterparts: 1) Innate™ tubers contained less asparagine, 2) more glutamine and 3) at least some degree of black spot bruise tolerance. The one exception being that conventional Atlantic already displays some black spot bruise tolerance. All of the events were free of detectable vector backbone DNA. Reduced asparagine contributes to significant reductions in acrylamide after heating, in all events, even if the insert contained only the *Asn1/Ppo5* silencing cassette and lacked the R1/PhL silencing cassette that would contribute to lower reducing sugars.

All insertions represent simplex insertions into tetraploid loci. This heterozygote genotype is commonly referred to as AAAa, whereby "a" represents an allele carrying the insertion, and "A" stands for lack of the insertion. Simplex insertions are unlikely to cause expression of recessive phenotypes, which usually require homozygote insertions (knock-out of all "A" dominant alleles).

It is possible but not likely that a promoter element positioned within an insertion could function as a long-distance enhancer or promoter for a neighboring open reading frame. Even if the expression of open reading frames adjacent to insert promoters had occurred, it would be rare that this would result in a functional and unwanted protein. If an insertion interrupted the expression of an essential gene, the interruption would be expected to disrupt the growth of the plant or change the composition of the plant tissues. The combined tests for molecular structure show that the potato events have resulted from the addition of genetic material from a donor organism where the material is well characterized and contains only non-coding regulatory regions of DNA.

5. Expression of the Gene Products

The DNA insert used to transform these ten potato events contains two expression cassettes designed to lower the transcript levels of endogenous genes. The first cassette contains two fragments of the asparagine synthetase-1 (*Asn1*) gene (404-bp) and the polyphenol oxidase-5 (*Ppo5*) gene (143-bp), inserted as inverted repeats between the convergent-oriented promoters of the ADP glucose pyrophosphorylase (*Agp*) and granule-bound starch synthase (*Gbss*) genes. The second cassette contains fragments of the promoters of the starch associated genes *R1* (531-bp) and phosphorylase-L (*PhL*) (508-bp), operably linked to the same regulatory elements used for the first cassette.

Transcript levels for the *Asn1* and *Ppo5* genes were reduced in the tubers, stolons, and, in some cases, other tissues of all potato events as expected (**Appendix 5. Efficacy and Tissue-Specificity of Gene Silencing**). It was also expected that transcript levels for the *PhL* and/or *R1* genes would be reduced in tubers, stolons, and, in some cases, other tissues of the potato events F10, F37, E12, E24, J3, J55, H37 and H50.

It is possible that these intended changes could trigger unintended effects because free asparagine and glutamine are signals of organic nitrogen status that regulates gene expression (Gutiérrez *et al.* 2008), while glucose stimulates the formation of abscisic acid and auxin while suppressing cytokinin production (Ramon *et al.* 2008). However, if unintended changes did occur, studies described in this petition demonstrated that they have no consistent impact on plant agronomics or tuber composition.

Previous studies have shown that *Ppo* gene silencing lowers the amount of protein to levels that are undetectable by Western blot analysis (Llorente *et al.* 2011). Similarly, silencing of the *R1* gene diminished accumulation of a ~160kDa protein that is at least partially bound to starch granules (Lorberth *et al.* 1998).

The inserted DNA in all 10 events included in this petition functions by silencing the genes involved with production of asparagine, polyphenol oxidase, and reducing sugars. By design, the events contain only non-coding regulatory sequences. Multiple studies included in this petition show the material to be well characterized and not associated with plant pest traits.

6. Efficacy and Tissue-Specificity of Gene Silencing

The purpose of these studies was to show the effectiveness of gene silencing based on RNA expression as measured through Northern blot analyses. Gene silencing methods were employed to lower the activity of the Asn1, Ppo5, PhL, and R1 native proteins. Transcript levels rather than protein amounts were evaluated to link new phenotypic traits to changes at the molecular level. For this purpose, RNA was isolated from various tissues of the events and their untransformed counterparts and used to carry out Northern blot analyses (see **Appendix 5. Efficacy and Tissue-Specificity of Gene Silencing**, with a summary in **Table 1**). Each letter (A, P, L, R) in **Table 6** indicates that silencing was confirmed, although the amount of silencing varied depending on the gene and tissue.

Innate™ technologies have given us the ability to selectively regulate genes in specific tissues rather than the entire plant. For example, strong silencing of the *Asn1* gene involved in ASN (asparagine) formation in potato tubers was desired, yet such changes in leaves and stems might adversely affect growth. The promoters used to drive gene silencing, the Agp promoter and the Gbss promoter, have been well characterized and are known to be highly active in tubers and stolons and less active in photosynthetically-active tissues and roots (Nakata *et al.* 1994; Visser *et al.* 1991). Therefore, silencing was predicted to be stronger in tubers and stolons, and less pronounced in leaves, stems, roots, and flowers, as shown in **Table 6**.

Table 6. Summary of Down-Regulated Genes in Different Tissues

Event	Tubers[1]	Stolons[1]	Roots[1]	Stems[1]	Leaves[1]	Flowers[1, 2]
F10	A P R	A L R	A P R	P	A	A
F37	A P R	A L R	A P R	A P		A R
E12	A P L R	A L R	A P	P		A
E24	A P L R	A P L R		A P		A
J3	A P L R	A R	A	A P		A
J55	A P L R	A L R	A	P		A
J78	A P	A		P		A
G11	A P	A P		R	R	A
H37[3]	A P L R	L R	P	P	R	
H50[3]	A P L R	A P L R	A P R	A P	R	

[1]A = *Asn1*, P = *Ppo*5, L = *PhL*, R = *R1*. Letters in table indicate down-regulated gene expression by tissue.

[2]The partially down-regulated *Asn1* gene expression might alter the amino acid composition of the flowers. Such effects will be limited to a reduction in ASN and an increase in GLN. Since ASN and GLN are similar non-essential amino acids, changes in the levels of these compounds is not expected to affect the quality of petal, nectar, and pollen as feed for insects or other organisms.

[3]Events derived from variety H do not produce flowers.

The targeted four genes (*Asn1, Ppo5, PhL, and R1*) are partially silenced in tubers of most events, except for J78 and G11 which do not contain the cassette for silencing the *PhL/R1* genes (**Table 6**). In addition, the *PhL* gene did not appear down-regulated in tubers of the Ranger Russet events, F10 or F37. For some events, E24, and H50, all four genes were also down-regulated in stolons. The two events J78 and G11, which contain a truncated DNA insert with a functionally active *Asn1*/*Ppo5* silencing cassette, produced tubers that contained reduced transcript levels for the *Asn1* and *Ppo5* genes only, since they did not contain the *PhL/R1* silencing cassette. Overall, more down-regulation of the four targeted genes occurred in tubers and stolons than in the roots, stems, leaves, or flowers (**Table 6**).

Event G11, which does not contain the PhL/R1 promoter silencing cassette, displayed reduced R1 transcript levels in leaves and stems. This unexpected change may be a consequence of DNA integration or somaclonal variation (Skirvin *et al*. 1993).

The expression studies showed that silencing was most effective in tubers and stolons, as predicted with the chosen promoters, Agp and Gbss. In addition, silencing was predictable based on the molecular characterization that showed all events contained the *Asn1* and *Ppo5* genes, but two events, J78 and G11 events, did not contain the *R1* and *PhL* genes, and therefore did not show silencing of those genes in tubers.

Considering the potato events in this study, expression of the targeted genes would provide evidence that the desired phenotype with low asparagine, reduced black spot, and lowered reducing sugars had been achieved. It could provide information relevant to the plant pest risk assessment, for example, all Innate™ potatoes in the study had reduced or similar expression to the controls. Thus, the altered expression is unlikely to affect weediness or other plant pest characteristics. Also, with these events, the expression studies pertain to endogenous proteins rather than new proteins that could require a safety evaluation, such as those associated with herbicide tolerance or insect resistance.

Additional evidence for the safety of these potato events compared to the untransformed controls is illustrated in the agronomy and composition studies. It can therefore be concluded that these modified expression levels do not impact plant agronomics or influence tuber composition. Thus, the expression studies provide additional evidence that the Innate™ potatoes are not plant pests and the integrated genetic material contains only non-coding regulatory regions that are well characterized, and are as safe as untransformed potatoes.

7. Agronomic Performance, Disease, and Efficacy of Traits

The purpose of the agronomic trials was to confirm that the Innate™ potatoes had equivalent phenotypes compared with their untransformed controls when grown at multiple locations representing the major areas for potato production in the US. Observations throughout the growing season allowed for a thorough assessment of growth, disease and pest susceptibility, and measurements of tuber yield and quality. The field assessments aided in the selection of the events that contained the intentionally incorporated new traits and maintained all benefits of the conventional parent varieties. These assessments also made it possible to ensure that the transformations had not resulted in the introduction of unintended effects associated with weediness or pest-like characteristics.

After completing the agronomic trials, potato tubers were tested for evidence of the efficacy of the traits, including an assessment for black spot bruise and reducing sugars. In addition, after processing into french fries or potato chips, the potatoes were tested for acrylamide. A brief summary of the acrylamide results will be presented here with the supporting details available in **Appendix 9. Compositional Analyses**.

7.1. Agronomy and Phenotypic Evaluation

This summary includes agronomic evaluations of 10 events, representing 5 varieties, at geographically distinct sites that represent most of the main production areas for potatoes destined for fry and chip production in the USA (**Appendix 6. Field Performance and Tuber Evaluations**). All agronomic trials were conducted under Biotechnology Quality Management System (BQMS) standards and USDA-APHIS compliance (For a complete listing of trials, see **Table 13** in **Section 14. Record of Field Test Reports**). Planting was carried out mechanically to facilitate subsequent harvests and to ensure that Innate™ potatoes were kept separate from unmodified material. The agronomic evaluations relate to both field observations and tuber assessments, both at harvest and during storage. **Table 7** shows which characteristics were evaluated. Some differences in data collected reflect differences in product use in processing, *e.g.*, Ranger Russet and Russet Burbank used for french fries and Atlantic, G, and H are used in the potato chip industry.

Table 7. Summary of Agronomic Observations

Characteristic	Number of Site Years[1]				
	Ranger Russet	Russet Burbank	Atlantic	G	H
Early Emergence	4	4	13	3	3
Final Emergence	13	4	4	3	3
Stems Per Plant	4	4	7	NA	NA
Plant Vigor	8-11	11	7-8	6	5
Foliage Color	8-11	11	14-15	6	5
Leaflet Size	8-11	11	14-15	6	5
Leaflet Curl	8-11	11	14-15	6	5
Senescence	3	3	6	NA	NA
Vine Size	NA	NA	NA	4	4
Vine Maturity Rating	7	7	9-10	5	5
Flower Color	2-4	3-4	7	NA	NA
Total Yield	11	11	14	3	3
% 4-6 oz.	9-10	8-10	NA	NA	NA
% 6-10 oz.	9-10	8-10	NA	NA	NA
% 10-14 oz.	9-10	8-10	NA	NA	NA
% >14 oz.	9-10	8-10	NA	NA	NA
Specific Gravity	9-10	8-10	14	3	3
High Sugar	9-10	8-10	NA	NA	NA
Sugar Ends	9-10	8-10	NA	NA	NA
Total Internal Defects	9-10	4	14	3	3
% U.S. #1	NA	NA	14	3	3
% Grade B	NA	NA	14	3	3
% Grade A	NA	NA	14	3	3
% Oversize	NA	NA	14	3	3
% Pickouts	NA	NA	14	3	3
Insect Stressors	3-5	5	7	1	1
Disease Stressors	3-5	5	7	1	1
Abiotic Stressors	3-5	5	7	1	1
Incidental Stressors	8	8	9	6	6

NA = characteristic not evaluated

[1] Total number of sites in 2009, 2010, and 2011 for each characteristic. Ranges indicate that not all lines of each variety were grown at every site.

The agronomic and ecological data that are presented in **Appendix 6** (**Field Performance and Tuber Evaluations**) show that over multi-year and multi-site field trials no variety specific differences leading to increased weed or pest potential were present. The studies were conducted with as much scientific rigor as possible to ensure product and trait safety. Statistical methods were employed to test for differences between the Innate™ varieties and appropriate controls. We used a step-wise approach to interpret any differences that were observed (both statistically significant and non-statistically

different). For each comparison between a test line (event) and the appropriate conventional control, the following logic was employed to assess the results. First, when p-values were available, statistical significance was determined for each comparison. If the p-value indicated no statistical significance, it was unlikely that there was a difference that would be biologically meaningful or indicative of increased pest potential. Next, if the p-value indicated statistical significance or the data did not support calculation of a p-value, the mean value of the test line was compared to the combined control range, which represents the minimum and maximum mean values of all conventional lines included in the experiments. If the value for the test line was within the combined control range, it was concluded that the test line exhibited characteristics within the natural variation of potatoes and that the difference was unlikely to be biologically meaningful or indicative of increased pest potential. If the value of the test line was outside the combined control range, further consideration was given to the difference in the context of agronomic impact and increased pest potential.

The combined control range for the agronomic characteristics used a total of 319 mean values from conventional Ranger, Burbank, Atlantic, G, and H varieties grown as controls in the field trials. For the yield and grading data for the Burbank and Ranger lines, the combined control range used a total of 170 mean values of the Burbank and Ranger conventional controls. For the yield and grading data for the Atlantic, G, and H lines, a total of 160 mean values of Atlantic, G, and H conventional controls were used to determine the combined control range.

Although some significant agronomic differences were observed between events and controls, most fell within the combined control range of the conventional varieties. For example, some differences in yield and tuber size were observed in the events, and these attributes will be investigated further in commercial trials. Thus, we concluded that no biologically meaningful differences that would contribute to increased weediness or pest potential were observed for any of the agronomic characteristics, yield and grading characteristics, or ecological interactions (namely plant-insect interactions, plant-disease interactions, and plant interactions with abiotic stressors) of the events compared to their untransformed controls **(Appendix 6. Field Performance and Tuber Evaluations**). There were no differences in phenotype and tuber composition (**Appendix 9. Compositional Analyses**) or disease susceptibility (**Appendix 8. Disease Susceptibility**). It can be concluded that the events are not different from their untransformed controls in terms of agronomy, tuber yield, and tuber composition. Therefore, the transformation of potato with pSIM1278 does not introduce characteristics that will encourage or enhance weediness or pest potential.

7.2. Disease susceptibility and polyphenol oxidase

Polyphenol oxidase enzymes (Ppo) are found in most organisms including animals, plants, fungi and bacteria. Although much is known about the molecular biology of Ppo and its role in enzymatic browning, little is understood about the function of Ppo-mediated browning in plant physiology. Some have hypothesized that Ppos represent part of the plant's defense response against insects and pathogens. Considering that the present submission involves silencing of Ppo in tubers, we reviewed relevant literature and conducted studies relating to the disease response of the events.

Ppos are copper metalloenzymes which oxidize mono- and o-diphenols to o-diquinones by utilizing molecular oxygen (Thipyapong *et al*. 2004). Typically, Ppo activity is latent until the enzyme is released by disruption of the cell structure through forces like wounding and senescence. When cell membranes are damaged, Ppo enzyme is released and reacts along with oxygen molecules to produce quinones

(Thipyapong *et al.* 2004). The production of black and brown quinones is responsible for much of the interest in PPO in the post-harvest physiology of many fruit and vegetable crops.

It is common for multiple homologues or alleles to exist within species, each responsible for expression in different plant tissues. In potato, 6 genes encoding Ppo have been identified, tomato (*Lycopersicon esculentum*) possesses 7 PPO genes, and banana (*Musa acuminata*) is known to contain a Ppo family of at least 4 genes (Thipyapong *et al.* 2007; Mayer 2006). A number of researchers evaluated induced Ppo activity and response mechanisms to biotic stressors, in particular pests and pathogens (Steffens *et al.* 1994). Studies with biotech tomatoes found a positive correlation between high levels of Ppo in leaf tissue and increased resistance to pathogens and insect pests. In one study, three lines with higher Ppo expression were tested against the bacterial pathogen *Pseudomonas syringae* pv. *Tomato*, the causal agent of bacterial speck in tomato (Li and Steffens 2002). These lines showed enhanced suppression of disease symptoms and exhibited 15-fold fewer lesions per leaf area than controls. Although these results indicated a protective effect of Ppo, the mechanism for disease resistance remains unknown (Li and Steffens 2002).

In potato, impacts sustained during harvest and postharvest activities induce the release of Ppo from cell plastids, facilitating oxidation of phenols to quinones, and resulting in negative effects on quality and recovery in processing french fries and chips, as well as the marketability of fresh potatoes. A family of 6 genes encoding Ppo exists in potato, with one gene (*Ppo5*) being tuber-specific and the remaining 5 genes responsible for Ppo expression in other tissues. The tuber-specific *Ppo5* gene was down-regulated, resulting in reduced susceptibility to black spot bruise, as shown in the current petition for nonregulated status.

Researchers found that by down-regulating Ppo activity in potato *e.g.*, see Hakimi *et al*. 2006, disease symptoms of *P. infestans* were reduced, apparently corresponding to an increase in the plant's resistance to late blight. The authors proposed that tubers may also display enhanced disease resistance against certain other fungal pathogens that infect potato tubers, including *Rhizoctonia* (black scurf), *Fusarium* (dry rot), *Spongospora* (powdery scab) and *Alternaria* (early blight).

Enzymatic browning is an important reaction that occurs in the fruit of the apple (*Malus domestica*). In a study conducted by Valentines *et al.* (2005), the roles of enzymatic browning and lignification (the chemical strengthening of cell walls in response to pathogenic infection) as resistance mechanisms against *Penicillium expansum* were investigated in Golden Delicious apples. A significant increase in decay was observed following the treatment of peeled apples with a Ppo substrate which had induced higher Ppo activity levels. The Ppo enzyme, and particularly the browning process induced by treatment with Ppo substrate, may have contributed to increase decay and indicates that an overexpression of Ppo may lead to higher level of sensitivity towards the pathogen (Valentines *et al.* 2005).

Some researchers have proposed that enhanced Ppo may increase resistance to disease, while others claim that reduced Ppo could also increase resistance. Considering that some evidence exists for a relationship between Ppo and diseases, we chose to test the events for several important potato diseases and conclude that there is no impact on disease resistance or susceptibility with regards to the *Ppo5* gene as demonstrated in the remainder of this section.

7.3. Disease Incidence: Storage Studies

Throughout the 2009 cold storage period, pink and soft rot diseases were occasionally observed on both untransformed and Innate™ potatoes from the Canyon County, ID, and Bingham County, ID, field trial sites. In addition, *Pythium* leak disease was observed on some untransformed and Innate™ potatoes from Adams County, WA, and Bingham County, ID, for the 2010 cold storage period. These observed disease incidences were attributed to environmental soil conditions that favored infection of tubers and the relatively high presence of disease within the specific field trial sites.

7.4. Disease Incidence: Late Blight and Bacterial Soft Rot

Disease incidence was evaluated by intentionally infecting the events and their untransformed controls with the causal agents of late blight (*Phytophthora infestans*) and soft rot (*Erwinia carotovora*) and evaluating disease progression. Details regarding the following tests are included in **Appendix 8. Disease Susceptibility**.

Late Blight Testing with Tubers. Results of studies from the 2009 field trials showed an increase in late blight symptoms in tubers of Russet Burbank event E12 and a decrease in symptoms in Ranger Russet event F10 compared with their respective controls. In similar tests with multiple late blight strains inoculated onto tubers, we found event G11 to be less susceptible, H50 more susceptible, and H37 variable compared with controls. After seeing these mixed results in a laboratory setting, we conducted additional field testing in 2011, using only the Ranger Russet, Russet Burbank and Atlantic varieties.
Using tubers from the 2011 field season, event E12 was found to be less susceptible to late blight than controls. In addition, Atlantic event J3 was found to be more susceptible than Atlantic controls; however, J3 was similar to Ranger Russet and Russet Burbank controls. Ranger Russet events F10 and F37 did not differ from their controls.

Late Blight Foliage Testing. In a replicated field trial in 2011, potato plants were deliberately infected with the causal agent of *P. infestans*. Of the events in that trial (E12, E24, F10, J3, J55, and J78), the only significant difference was that event F10 was more resistant than control. This replicated field trial provides evidence that Innate™ events were no more susceptible to late blight than the untransformed controls.

Soft Rot Testing with Tubers. A test with tubers in 2009 showed a similar response in controls and all events tested. Further testing in 2011 showed that Atlantic events J3 and J55 were less susceptible than untransformed controls. Considering both studies, we conclude that the events have similar susceptibility to bacterial soft rot as the controls

The studies with late blight and bacterial soft rot confirm that silencing of the target genes did not enhance susceptibility to these common diseases. As with any new variety development, we would want to avoid modifications that enhance disease susceptibility. However, if any events showed slightly higher susceptibility to disease, it would not enhance the weediness or result in the creation of plant pests.

7.5. Evidence of Trait Efficacy: Reducing Sugars

Tubers contain large amounts of phosphorylated starch, some of which is degraded during storage to produce glucose and fructose. These reducing sugars react with amino acids to form Maillard products

including acrylamide when heated at temperatures above 120^{O} C. Two enzymes involved in starch phosphorylation are water dikinase R1 and phosphorylase-L (R1 and PhL*)*. The partial or complete silencing of the associated genes in tubers can limit the accumulation of sugars, thus decreasing the potential to produce acrylamide.

The amount of reducing sugars was assessed by taking samples from either fresh or stored potatoes (**Appendix 9. Compositional Analyses**). Many of the events had lowered levels of reducing sugars either at the time of harvest or after storage for 1 month (F10, F37, E12, E24, J3, and J55, J78), however, in most cases we did not observe significant differences after 2-5 months of storage. It was not expected that J78 would have lowered levels of reducing sugars because it was one of two events, J78 and G11, that did not contain the R1/PhL promoter silencing cassette. Tubers of the events G11, H37, and H50 contain the same amount of reducing sugars as tubers of their untransformed counterparts. The inability of the silencing construct to limit glucose/fructose formation in H37 and H50 may be due to the fact that the H variety is naturally low in glucose and fructose. Thus, we concluded that silencing of the promoters associated with the *PhL/R1* genes effectively lowered reducing sugars near the time of harvest in most events but these differences were not sustained throughout storage for 2-5 months. These data indicate that partial silencing of the *R1* and *PhL* genes contributes to lower reducing sugar levels and a lowered potential to form acrylamide.

7.6. Evidence of Trait Efficacy: Black Spot Bruise

Two different methods were utilized to measure the incidence of black spot bruise, one by catechol assay and the other by physically impacting tubers using a rotating drum (**Appendix 7. Black Spot Bruise Tolerance**). Tubers of events were shown to contain either very low (F37) or undetectable activity levels (all other events, except for Atlantic events), as visualized by a catechol assay. Atlantic events were not tested because Atlantic controls did not react with the catechol assay and appeared to display some black spot bruise tolerance. The barrel bruise test indicated undetectable levels of black spot bruise in event E12, at least a 5-fold reduction in levels for events F10 and F37, and about 2-fold reduced levels in events E24, H37, and H50. The black spot bruise tolerance of event G11 showed variability depending on site.

7.7. Evidence of Trait Efficacy: Reduced Asparagine and Acrylamide

Silencing of the asparagine synthetase gene resulted in average reductions of 69 to 78% free asparagine in the potato events (**Table 8**). The lower levels of asparagine which combines with reducing sugars in the Maillard reaction to form acrylamide, results in average reductions of 58 to 72% acrylamide in fries and chips (**Table 8**). Along with the reduction in free asparagine, we have observed an increase in free glutamine, a direct result of silencing the gene that codes for the asparagine synthetase enzyme that converts glutamine to asparagine (**Figure 6**).

Table 8. Differences in Asparagine and Acrylamide Between Controls and Events

Variety	Free Asparagine (ppm)	Percent Reduction from Control	Acrylamide (ppb)[1]	Percent Reduction from Control
Ranger Control	2351	0%	371.9	0%
F10	567.2	76%	150.6	60%
F37	642.5	73%	127.3	66%
Burbank Control	2421	0%	493.8	0%
E12	618.2	74%	162.4	67%
E24	591.9	76%	158.9	68%
Atlantic Control	2268	0%	842.7	0%
J3	516.2	77%	278.7	67%
J55	502.4	78%	233.4	72%
J78	551.1	76%	251.1	70%
G Control	3406	0%	1254.2	0%
G11	794	77%	421.5	66%
H Control	2805	0%	531.2	0%
H37	877.5	69%	223.0	58%
H50	672.7	76%	174.7	67%

[1]Before testing for acrylamide, Ranger Russet and Russet Burbank controls and events were made into french fries. Atlantic, G, and H varieties were processed into potato chips. All results were from tubers analyzed near the time of harvest.

Nitrogen assimilation into amino acids occurs in all organisms and involves numerous genes and enzymes. As the primary nitrogen transport compounds within the plant, the amino acids glutamine and asparagine play crucial roles for plant growth and development (McGrath and Coruzzi 1991; Urquhart and Joy 1981). In senescing leaves, protein hydrolysis produces ammonium (Masclaux-Daubresse *et al.* 2006). Therefore, toxic ammonium must be immediately re-assimilated into organic molecules through nitrogen cycling. First, ammonium is assimilated into the glutamine amide group. Next, glutamine transfers to the position of 2-oxoglutarate, yielding two molecules of glutamate caused by the concerted reaction of the glutamine synthetase gene (*GS*). Typically, the nitrogen incorporates into other amides and amino acids, including asparagine. The glutamine-dependent enzyme asparagine synthetase (*Asn1*) catalyzes the reaction that converts aspartate to asparagine, transferring the amide nitrogen group, and converting glutamine to glutamate. The asparagine biosynthesis pathway is represented in **Figure 6**. Using Innate™ technologies, the *Asn1* gene is silenced in potato tubers, thereby allowing glutamine to retain its amide group, and to remain as glutamine in the system. This explains the reason lower asparagine levels in Innate™ potatoes create a higher level of glutamine.

Figure 6. Biosynthesis of GLN and ASN in plants

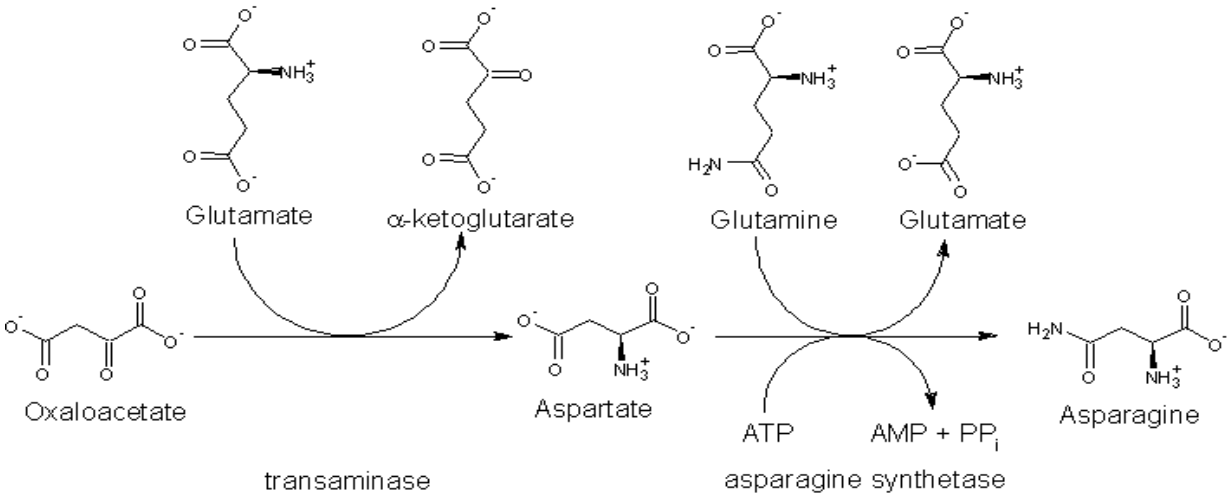


Figure 6 is from New World Encyclopedia, http://www.newworldencyclopedia.org/entry/Asparagine.

7.8. Survival in Fields

It is possible that Innate™ or conventional potatoes will persist in fields for several years after planting. In most Northern areas, potatoes are rotated with other crops which minimizes the survival in subsequent years and the chance of accidental mixing in subsequent potato plantings. However, growers who have planted Innate™ potatoes will be instructed to eliminate any potatoes persisting in the field before reverting to conventional potato production. Farmers will also be instructed not to sell waste potatoes from Innate™ plantings to small processors unless the trade restrictions with this material are clearly stated and the small processors are not engaged in potato export outside of North America.

After conducting field trials with potatoes, we routinely look for potatoes in the field the year following. We have not found any potatoes to persist in test plots from 2009 – 2011 trials after monitoring and destroying volunteers in 2010, 2011, and 2012 (to date as of this submission) (Summarized in **Appendix 10. Post-Harvest Monitoring**). These field trials consisted of small plots that are carefully monitored by researchers and represent a well-controlled environment. In large tracts of land typical of commercial potato growing operations, there might be a greater likelihood that potatoes could be overlooked. If potatoes were grown repeatedly year after year on the same ground, it would be more likely for contamination of a subsequent potato crop to occur. Extra precautions may need to be considered in those instances.

7.9. Conclusion on Agronomic Performance, Disease, and Efficacy of Traits

Transformation of potato with the DNA insert of pSIM1278 did not alter the agronomic characteristics of potatoes, and yielded the expected changes of reduced back spot bruise, lower levels of reducing sugars and reduced acrylamide potential (see **Table 9**). Reducing sugars were lowered slightly in most events, and would not be expected to be diminished in the events that did not contain the silencing construct. Significantly reduced asparagine contributed to reduced levels of acrylamide in fries and chips. The studies with late blight and bacterial soft rot confirm that silencing of the target genes did not enhance

susceptibility to these common diseases. However, if any events showed slightly higher susceptibility to disease, it would not enhance the weediness or result in the creation of plant pests.

Table 9. Traits Incorporated into Innate™ Potatoes

Event	Reduced Acrylamide Potential	Black Spot Bruise Tolerance	Reduced Accumulation of Glucose and Fructose
F10	Yes	Yes	Yes
F37	Yes	Yes	Yes
E12	Yes	Yes	Yes
E24	Yes	Yes	Yes
J3	Yes	PPO assay indicated some resistance in the Original Variety	Yes
J55	Yes	PPO assay indicated some resistance in the Original Variety	Yes
J78	Yes	PPO assay indicated some resistance in the Original Variety	Yes
G11	Yes	Yes (Variable depending on site)	No
H37	Yes	Yes	No
H50	Yes	Yes	No

Although some significant agronomic differences were observed between events and controls, most fell within the combined control range of the conventional varieties. For example, some differences in yield and tuber size were observed in events, and these attributes will be investigated further in commercial trials. Thus, we concluded that no biologically meaningful differences that would contribute to increased weediness or pest potential were observed for any of the agronomic characteristics, yield and grading characteristics, or ecological interactions (namely plant-insect interactions, plant-disease interactions, and plant interactions with abiotic stressors) of the events compared to their untransformed controls. It can be concluded that events are not different from their untransformed controls in terms of agronomy, tuber yield, and tuber composition. Therefore, the transformation of potato with pSIM1278 does not introduce characteristics that will encourage or enhance weediness or pest potential.

8. Compositional assessment

The compositional assessment studies, evaluating proximates, vitamins, amino acids, minerals, sugars, and glycoalkaloids, were conducted on Innate™ potatoes to 1) show equivalence to the untransformed controls, 2) compare the Innate™ potatoes to literature ranges, where applicable; and 3) show that there were no changes in potential toxins. Ultimately, results of the composition studies showed food safety equivalence between the events and the untransformed controls.

8.1. Proximates, Vitamins, Minerals, and Glycoalkaloids

These analyses were conducted to confirm that composition of events remained within the normal levels for potato and would have equivalent food quality, feed quality, and safety when compared to untransformed potatoes (**Appendix 9. Compositional Analyses**). We also tested for the effectiveness of gene silencing and expected to find lower amounts of asparagine and, in most cases, lower amounts of glucose and fructose compared to untransformed controls. The compositional assessments determined the amounts of 1) moisture, protein, total fat, ash, crude fiber, carbohydrate, and calories; 2) vitamins

B6, B3, and C; 3) minerals copper, magnesium, and potassium; 4) glycoalkaloids; 5) free amino acids; 6) total amino acids; and 7) reducing sugars for tubers collected from events grown in potato-growing areas of the United States. For all the nutrients listed above, the goal was to demonstrate that events contained equivalent amounts compared with the untransformed controls. Along with agronomic data, the compositional analyses helped determine whether the Innate™ potatoes exhibited compositional changes that might impact plant pest risk or food and feed safety.

Statistical analyses of nutrient composition were conducted to test for differences in the analyte mean values between each of the events and their untransformed controls. A complete description of statistical methods can be found in **Appendix 9. Compositional Analyses**. Either the P-value or adjusted P-values are provided, whereby differences $\leq$ 0.05 were considered statistically significant. Further context for data interpretation was provided through the use of tolerance intervals and published literature.

The following varieties, all grown in the various field trial sites, were used to calculate tolerance intervals: Snowden, Chieftain, Red Norland, Ida Rose, Russet Burbank, Ranger Russet, Atlantic, G, and H. The tolerance intervals were calculated to contain, with 95% confidence, 99% of the values contained in the population. This statistical 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 of all significant differences between events and controls are summarized in **Table 10** for Ranger Russet and Russet Burbank and in **Table 11** for Atlantic, G, and H varieties. Most of these differences; reduced free ASN and total ASP + ASN, increased free GLN and total GLU + GLN, reduced acrylamide, and lowered reducing sugars were expected because of the intended gene silencing. Other significant differences included decreased free VAL (J3, J55), decreased free LYS (H37), increased free ARG (F37), increased total LYS (G11), increased total PRO (G11), increased Vitamin C (F10, F37), increased niacin (F10, F37), decreased pyridoxine (J3, J55), decreased sucrose (E12, E24), and increased sucrose (H37). Both the intended changes and these observed differences fell within the tolerance intervals or combined literature values, and therefore were considered to be within normal ranges for potatoes.

It's important to note that for events E12 and E24, the free ASN levels were not statistically different from controls at the adjusted $p \leq .05$ level. However, these events showed a 74 to 76% reduction in free ASN. Such large differences were sometimes not statistically significant, which was a direct result of using the correction for False Discovery Rate (Benjamini and Hochberg 1995). These reductions were of practical significance and are considered similarly to the other events regarding this trait.

Note that free amino acids would not normally be part of a compositional assessment, however, they were included because the quantity of free asparagine is important to show the efficacy of silencing the *Asn1* gene for the purpose of reducing acrylamide. Many of the free amino acids are found at such low levels in potatoes that values barely exceed the detection limit. For the purpose of assessing the nutritional equivalence of the events, we recommend that the focus should be on comparing total amino acids to controls.

The most important changes between Innate™ tubers and their untransformed controls relates to reduction of the amino acid asparagine (ASN) and reducing sugars. Reduced amounts of free ASN and

reducing sugars in the Innate™ potatoes resulted in lowered acrylamide after heat processing compared to the conventional potatoes (see **Tables 10** and **11** as well as **Appendix 9. Compositional Analyses**).

Table 10. Statistical Differences in Composition Data: Events F10, F37, E12 and E24

Variety	Event	Attribute	Difference Expected?	Comments
Ranger Russet	F10	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Increased Niacin (B3)	No	Within TI and CLR
		Increased Vitamin C	No	Within TI and CLR
Ranger Russet	F37	Decreased Free ASN	Yes	
		Increased free ASP	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Increased Vitamin C	No	Within TI and CLR
		Increased Niacin (B3)	No	Within TI and CLR
		Increased free ARG	No	Within TI and CLR
Russet Burbank	E12	Decreased Free ASN	Yes	74% reduced, not significant
		Decreased Total ASN+ASP	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Sucrose	No	Within TI and CLR
Russet Burbank	E24	Decreased Free ASN	Yes	76% reduced, not significant
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Sucrose	No	Within TI and CLR

TI = tolerance interval
CLR = combined literature range

Table 11. Statistical Differences in Composition Data: Events J3, J55, J78, G11, H37 and H50

Variety	Event	Attribute	Difference Expected?	Comments
Atlantic	J3	Decreased Free ASN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Pyridoxine (B_6)	No	Within TI and CLR
		Decreased Free VAL	No	Within TI and CLR
Atlantic	J55	Decreased Free ASN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Pyridoxine (B_6)	No	Within TI and CLR
		Decreased Free VAL	No	Within TI and CLR
Atlantic	J78	Decreased Free ASN	Yes	Adjusted P = .0548
		Decreased Reducing Sugars	No	Glucose and Fructose
		Reduced Acrylamide	Yes	
Variety G	G11	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Reduced Acrylamide	Yes	
		Increased Total LYS	No	Within TI and CLR
		Increased Total PRO	No	Within TI and CLR
Variety H	H37	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Increased Sucrose	No	Within TI and CLR
		Reduced Acrylamide	Yes	
		Decreased Free LYS	No	Within TI and CLR
Variety H	H50	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Reduced Acrylamide	Yes	

TI = tolerance interval
CLR = combined literature range

For the compositional assessment, all events were compared with their respective controls mostly at the time of harvest. In addition, the potatoes were tested for reducing sugars and acrylamide, following storage (**Appendix 9. Compositional Analyses, <u>Storage Studies: Reducing Sugars and Acrylamide</u>**). Many of the events had lowered levels of reducing sugars either at the time of harvest or after storage for 1 month (F10, F37, E12, E24, J3, and J55, J78), however, in most cases we did not observe significant differences after 2 – 5 months of storage. Thus, we concluded that silencing of the promoters

associated with the *PhL/R1* genes effectively lowered reducing sugars near the time of harvest but these differences were not sustained throughout storage for 2-5 months.

During storage of up to 7 months, acrylamide levels in most events were consistently lower ($p < .0001$) than controls, although there were exceptions with the G and H varieties. In event G11, we observed a mean of 2,050 ppb compared with 1,769 ppb for the control after 1 month of storage. This was unusual and throughout the rest of the study, acrylamide levels in event G11 were always much lower than controls (46 to 58% reduction at months 5 and 7). For variety H, acrylamide levels were always lower in events H37 or H50, but not statistically significant in months 5 and 7. Overall, the modifications from insertion of pSIM1278 resulted in dramatic reductions in acrylamide that persisted throughout the typical storage periods.

This research also confirmed that the events are as safe as controls and did not contain increased levels of natural glycoalkaloids, which are toxins commonly found in Solanaceous crops, including potato. The principal glycoalkaloids in potatoes are α-solanine and α-chaconine. For food safety purposes, an upper limit for glycoalkaloid content of 20mg per 100g of potato is generally accepted.

The Innate™ potato events F10, F37, E12, E24, J3, J55, J78, H37, H50 and their corresponding untransformed controls had mean glycoalkaloid ranges from 5.7 to 11.3 mg/ 100g (**Table 12**); all below the 20mg/ 100g safety limit described by Sinden (1987). For one event, E24, the range of values extended to 33.81, beyond the desired limit of 20 mg/ 100 g. Although a cause was not identified, this could be from mis-handling and excessive exposure to light (Percival 1999). Since the mean value was close to the control, we would not expect that glycoalkaloid production was related to the modification.

The Innate™ potato event G11 had mean glycoalkaloids of 20.3 mg/100g and untransformed controls contained 19.8 mg/100g (**Table 12**). The higher mean levels of glycoalkaloids in the G event and untransformed controls is attributed to additional handling at the storage sites and thus, increased exposure to light and temperature flux (Percival 1999). Based on the lack of differences between the events and controls, there would not be a safety issue as a result of the introduction of the reduced acrylamide potential and low black spot bruise traits in Innate™ potatoes. All glycoalkaloid levels were within the normal ranges for potato, when compared with the tolerance intervals or combined literature ranges.

Table 12. Glycoalkaloids (mg/100 g)[1] in Ranger Russet, Russet Burbank, Atlantic, G, and H Varieties

Variety	Total Glycoalkaloids (mg/100 g)				
	Mean	Range	P-Value	TI	CLR[2]
Ranger Control	6.704	3.06-10.23		0.000-33.05	3.20-210.4
F10	6.979	2.84-9.51	0.8285		
F37	6.805	4.28-9.42	0.9812		
Burbank Control	7.404	2.74-18.59		0.000-33.05	3.20-210.4
E12	5.678	1.69-10.53	0.1707		
E24	6.845	1.45-33.81	0.8174		
Atlantic Control	6.274	2.09-11.40		0.000-33.05	3.20-210.4
J3	5.723	1.62-11.67	0.2393		
J55	5.864	1.83-11.12	0.4833		
J78	6.084	1.65-14.27	0.7198		
G Control	19.82	6.17-44.40		2.830-94.46	2.20-154.20
G11	20.32	5.81-55.3	0.9153		
H Control	10.65	5.96-21.07		1.470- 303.5	2.20-154.2
H37	10.76	5.41-17.25	0.8951		
H50	11.30	6.23-22.81	0.4379		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. P-values indicating significant differences with controls would be underlined.
[2]Literature ranges from Kozukue *et al*. (2008).

We would not expect to find novel amino acid sequences or proteins in the events because the expression cassettes consist only of non-coding DNA. There are two expression cassettes in the transferred DNA insert. One comprises an inverted repeat of fragments of asparagine synthetase-1 gene and the polyphenol oxidase-5 gene between the Agp and Gbss promoters. The other one is comprised of fragments of the promoter of the starch associated gene (R1) and the phosphorylase-L gene linked to the same Agp and Gbss promoters as the first cassette. The pool of mRNA produced by these two cassettes from either a fragment of promoters or a part of genes is unlikely to be further processed to form any amino acids or proteins. Instead, all mRNA produced by the DNA insert is processed using RNAi machinery in plant cells to silence the targeted genes: destroy mRNA of the targeted genes (Asn1, PPO) and prevent translation to form active gene products such as a protein. Therefore no changes to amino acid sequences or proteins are found with Innate™ potatoes.

8.2. Allergenicity of Innate™ Potatoes

The potato events described in this petition have the same composition compared to untransformed controls. Therefore, issues with allergies would not be expected above what individuals with potato allergies would normally have. Some allergies have been detected in children as a result of patatin contained in potatoes.

Patatin is a storage glycoprotein that displays lipase activity and makes up about 40% of the soluble protein in tubers (Mignery *et al.* 1988). Allergic reactions to processed potatoes are considered to be

very uncommon and have been reported for children only (De Swert *et al.* 2002; De Swert *et al.* 2007), with patatin (Sol t 1) identified as the major allergen involved in this reaction (Astwood *et al.* 2000). Because potato protein naturally contains a relatively large proportion of patatin, those who are allergic to potatoes would avoid them and changes to patatin levels would not alter that behavior.

8.3. Conclusions of Compositional Assessment

The intentional changes in ASN and reducing sugars accomplished through transformation of various potato varieties with the DNA insert of pSIM1278 resulted in lower acrylamide levels in french fries and potato chips. These modifications did not alter the quality of potato as food because (1) altered ASN and GLN levels are still within the normal range for potato, and (2) any impact on nutritional quality would be minimal from the observed differences in the levels of ASN, ASP, GLU and GLN because as non-essential amino acids they can be synthesized by the body. Unintentional changes in the amounts of specific amino acids, vitamin C, pyridoxine and vitamin B3 were minor and inconsistent among varieties, while resulting in levels that fell within normal ranges for potato. While one of the varieties (G) had higher levels of glycoalkaloids than expected, the events were no different from untransformed controls and were within the literature ranges reported for potato varieties. Therefore, it can be concluded that tubers from the events are as safe for consumption in food and feed as the untransformed controls.

9. Environmental Assessment of Introduction

After a thorough battery of studies to investigate the weediness potential and food and feed safety of Innate™ potatoes, the evidence submitted demonstrates that the Innate™ technologies used to introduce native traits is unlikely to harm humans, animals, or the environment.

The results of the composition analyses as described in **Appendix 9. Compositional Analyses** show overwhelming evidence of a lack of differences between Innate™ potatoes and the untransformed controls when tested for proximates, minerals, and glycoalkaloids.

The agronomic trials confirmed that the Innate™ potatoes had similar phenotypes compared with the untransformed controls when grown at multiple locations representing the major areas for potato production in the US. Observations throughout the growing season allowed for a thorough assessment of growth, ecological interactions including potential altered susceptibility to insects and diseases, and measurements of tuber yield and quality. The field assessments aided in selection of the most viable plants that contained the intentionally incorporated new traits and maintained all benefits of the conventional parent varieties (from among a larger number of events). Additionally, the field studies were used to ensure that the transformations had not resulted in the introduction of unintended effects associated with weediness or increased pest potential.

After completing the agronomic trials, potato tubers were tested for evidence of the efficacy of the traits, including an assessment for black spot bruise and reducing sugars. In addition, after processing into french fries or potato chips, the potatoes were tested for acrylamide.

Proper assessment of a biotech crop includes testing that identifies any potential negative impact on the environment. The concept of familiarity has been widely used to describe the interactions that a transformed plant may have in nature by considering the biology of the crop, the introduced trait, the receiving environment, and the interactions with all of these factors (Hokanson *et al.* 1999). This concept allows regulatory decision-makers to draw upon past experience with introduction of plants

into the environment and compare genetically engineered plants to their non-engineered counterparts. In the case of Innate™ potatoes, all of these factors were considered with respect to familiarity. Beyond the environmental factors it is also important to consider the utility of the trait. In this case, the trait reduces the level of acrylamide after frying, which can have a positive effect on human health while maintaining the typical characteristics of a potato *e.g.*, not changing farming practices or preparation of potatoes for human consumption.

9.1. Potential for Gene Transfer and Outcrossing

As previously discussed, the risks associated with accidental gene transfer in potatoes through outcrossing would be minimal for several reasons: 1) potatoes are almost always clonally propagated using "seed potatoes"; 2) a high percentage of fertile potatoes will be self-pollinated and are not frequented by honeybees due to a lack of nectar; 3) pollen transfer tends to be limited to about 20 meters which could be controlled easily in commercial fields; and 4) it is unlikely that true Innate™ seeds would grow into mature potatoes since potato seeds are not saved and propagated in the typical farming operation.

In the unlikely event that outcrossing was to occur between Innate™ and untransformed plants in the field, the impact would be negligible to future plantings since tubers rather than seed are removed from the field. If seedlings did arise from an outcrossing event, establishment would be nearly impossible since potatoes are grown in rotation and are poor competitors compared to other crop and weed species (Love 1994; CFIA 1996). If seedlings were to establish after harvest, they would be easily identified and eliminated as part of the standard agricultural practices in potato production. In either case, seedlings could be identified and eliminated to prevent them from entering the commercial stream.

In the unlikely case of growing TPS seed near the commercial Innate™ fields, a separation of 20 meters would be required to avoid outcrossing with potato events (Conner and Dale 1996).

9.2. Inter-Species Pollination and Hybridization Potential

The tetraploid species *Solanum fendleri* and diploid *Solanum jamesii* are the only wild species that grow within the borders of the United States (Bamberg *et al.* 2003; Spooner *et al.* 2004*).* These species mostly occur outside of the major potato growing regions in Texas, New Mexico, Utah, Colorado, and Arizona (see **Figure 2**). These species reside in dry forests, scrub desert, and sandy areas at altitudes of 5,000 to 10,000 feet, well isolated from most commercial production areas (Bamberg and del Rio 2011a). Therefore, pollen flow from commercial events to wild species is extremely unlikely simply because of the geographic location.

All Texas records in the IPD database are from the far western part of the state in El Paso, Culbertson and Jeff Davis Counties, where potatoes are not grown commercially. The two *S. fendleri* and one of the *S. jamesii* records are from the Davis Mountains, and one of the three *S. jamesii* entries is from Guadalupe National Park (IPD 2011; National Atlas 2004b; Hall *et al*. 2000).

The Colorado records in the IPD database are from mountainous locations very near the New Mexico border. Spooner *et al.* (2004) report that records of *S. jamesii* from natural habitats in Colorado are limited to the extreme southwestern and southeastern parts of the state. The San Luis Valley, a major

potato production area, lies between the mountainous regions where the wild potatoes were found (Bamberg *et al.* 2003; National Atlas 2004a; Spooner *et al.* 2004).

While there appears to be minimal, if any, overlap geographically between cultivated and wild potatoes in the U.S., there is a possibility that a few wild potato plants may be growing near potato fields (Love 1994). Spooner *et al.* (2004) describe *S. jamesii* habitat in the U.S. as among boulders on hillsides, sandy alluvial stream bottoms, in gravel along trails or roadways, rich organic soil of alluvial valleys, sandy fallow fields, grasslands, juniper-pinyon scrub deserts, oak thicket, coniferous and deciduous forests at elevations between 4,500 to 9,400 feet. They describe *S. fendleri* habitat similarly, and at elevations between 4,700 to 11,200 feet.

Based on conversations with Bamberg, Love (1994) reported that no one has ever reported finding hybrids between native and cultivated potatoes in the U.S., although gene transfer has been accomplished using special laboratory techniques (Love 1994). Love concluded that, based on the barriers that exist, including geographic isolation (as described above), endosperm imbalances, and multiple ploidy levels, natural hybridization is highly unlikely, and introgression (cross hybridization over multiple generations) impossible or at least highly improbable (Love 1994). More recently, the US EPA has concluded that, based on its review of the scientific literature, successful gene introgression between native and cultivated potatoes in the U.S. is virtually excluded due to constraints of geographical isolation and other barriers to natural hybridization. These barriers include incompatible (unequal) endosperm balance numbers that lead to endosperm failure and embryo abortion, multiple ploidy levels and incompatible mechanisms that do not express reciprocal genes to allow fertilization to proceed. No natural hybrids have been observed between these species and cultivated potatoes in the U.S. (US EPA 2011).

9.3. Weediness Potential

As previously described, weediness is a term used to describe the ability of a plant to become a weed (survive and thrive) outside of cultivation. The Innate™ modifications described in this petition are unlikely to increase the weediness of potato because the incorporated traits (reduced free-asparagine, black spot bruise tolerance, and reduced sugars) are not associated with weediness and will not help the plants thrive outside of cultivation. Multiple field trials of 10 events did not provide any evidence for altered growth characteristics such as accelerated tuber sprouting, increased plant vigor, increased tuber set, and delayed senescence or other key agronomic characteristics associated with survival outside of cultivation. Additionally, all fields were monitored for 2 years after study completion for volunteer activity as required by USDA compliance. Through 3 years of field studies and up-to-date volunteer monitoring of every site, no differences have been observed that would lead us to believe that the events have properties that would increase the survivability compared to conventional potatoes (see **Appendix 10. Post-Harvest Monitoring)**. Due to modern agricultural practices it is highly unlikely that potatoes would persist in a field from one crop cycle to the next, particularly since potatoes are typically grown as a rotational crop.

9.4. Endangered Species Considerations and Impact on Biodiversity

Endangered Species

Based on an assessment of threatened and endangered species in potato cropping systems, and considering the safety and compositional data of Innate™ potatoes, it is highly unlikely that there will be any effect on endangered species, the environment, or non-target organisms. Since these potatoes

introduce nothing new to the environment, *e.g.*, no new proteins or genes, they would have the same effect on endangered species as any other potatoes. Innate™ varieties are likely to be planted in areas that are already growing potatoes, and would not result in a significant expansion of acres.

Natural Biodiversity

The modifications described in this petition were intended to enhance the quality, not agronomic characteristics, of potato by reducing the expression of four native genes. Thus, we reduced the plant's ability to develop black spot bruise, store asparagine, and convert starch to sugars. Because no new pesticides are expressed, and no natural defense mechanisms have been activated, the modified potatoes have no new properties to enhance their weediness or pest resistance. The incidence of disease symptoms identified during growth and storage were similar for Innate™ and untransformed control potatoes. The Innate™ plants show no tendency to be more invasive. Invasiveness is not a trait associated with any potatoes and therefore not associated with silencing the genes affecting potato quality. These changes in potato quality have no more impact on biodiversity and endangered species than any other commercial potato varieties.

9.5. Effects on Current Agronomic Practices in Potato

Field trials with multiple potato events over three different crop years have indicated that no differences will be needed in the agronomic practices used to grow the Innate™ potatoes. The incorporated traits are not expected to affect agronomic practices. All events considered for this submission were selected to exhibit similar phenotypic characteristics to the control varieties when grown using standard industry practice. There were no differences in disease susceptibility that would require additional treatments nor did the potatoes have different nutrient requirements that would alter fertilizer programs. Planting, cultivation, management and harvesting processes were not affected by the incorporated traits. (see **Table 3** for a typical agronomic input scenario in the Northwestern United States).

9.6. Unintended or Non-target Effects

The lack of insecticidal traits in Innate™ potatoes would mean that all insects would be considered non-target. There are a large number of insects that feed on potato leaves and other insects that feed on pests. Many of these insects, including the Colorado potato beetle, potato aphid, European corn borer, potato leafhopper, and potato psyllid are considered pests. The events have not been modified to display any new pesticidal activities and do not contain new pesticide-expressing genes. No differences were observed in the field trials for insects or other animals interacting within the potato ecosystem proving a lack of pest potential to other organisms. The only modifications concerned the intended effects of reduced transcript levels for four quality-associated genes, mainly in tubers and stolons. During the three seasons of field releases, the observations and measurements showed no differences in ecological interactions (namely plant-insect interactions, plant-disease interactions, and plant interactions with abiotic stressors) between the events and the control varieties (**Appendix 6. Field Performance and Tuber Evaluations**), supporting an absence of unintended effects.

9.7. Summary of the Environmental Assessment of Introduction

The modifications described in this petition were intended to enhance the quality, not agronomic characteristics, of potato by inactivating four native genes. Planting, cultivation, management and harvesting techniques were not affected by the incorporated traits. The Innate™ varieties are likely to be planted in areas that are already growing potatoes, and would not result in a significant expansion of

acres. The modification described in this petition is highly unlikely to increase the weediness or invasiveness of potato because the incorporated traits (reduced free-asparagine, black spot bruise tolerance, and reduced sugars) do not play a role in survival, dispersal, or ecological competitiveness. Field trials over multiple years with 10 events did not provide any evidence for altered growth characteristics such as accelerated tuber sprouting, increased plant vigor, increased tuber set, or delayed senescence. No new pesticides are expressed, and no natural defense mechanisms activated, and thus modified potatoes are unlikely to display enhanced weediness or pesticidal properties. Therefore, the potential to impact insects and other non-target organisms, weed or disease susceptibility, endangered species or biodiversity is negligible for Innate™ potatoes.

The data presented in this petition demonstrate that introduction of Innate™ potatoes will have a similar environmental impact when compared to untransformed potatoes. Use of Innate™ technologies and the resultant transformed plants, and their tuber products, satisfy the statutory requirements for nonregulated status in that our transformants have no pesticidal traits and are not plant pests. To conclude, we feel that the plants containing these traits, are not plant pests and have resulted from the addition of genetic material from a donor plant where the integrated genetic material contains only non-coding regulatory regions, that are well characterized, and are as safe as untransformed potatoes. The evidence presented in this submission demonstrates that the deregulation and cultivation of Innate™ potatoes is highly unlikely to cause any adverse environmental or biological impacts as a result of their cultivation.

10. Crop Introduction and the Potato Industry

10.1. Industry Need for Potatoes with Reduced Acrylamide Potential

Based on a recent notice (Federal Register 2009), FDA is considering issuing guidance for industry on the reduction of acrylamide levels in food products. The introduction of Innate™ potatoes with low acrylamide potential would provide potatoes that are largely indistinguishable from existing varieties. The reduction in asparagine and sugars using Innate™ technologies and the resulting reduction in acrylamide upon heating will address industry needs with respect to the proposed FDA guidance.

In addition, recent litigation in the state of California resulted in legal settlements with restaurant chains, and the retail french fry and potato chip manufacturers regarding acrylamide. The settlements required manufacturers of retail fries and potato chips to reduce the level of acrylamide in their products. Potato processors affected by these rulings in California could be motivated to adopt the low acrylamide, Innate™ potato events.

The low acrylamide potatoes will provide a safer option for all sectors of the potato processing market and thus should increase the demand for these Innate™ potatoes. The benefits resulting from the modifications could result in increased market share, particularly for Ranger Russet and Atlantic. The reduction of sugars and black spot bruise in Ranger Russet may increase the popularity of that variety.

10.2. Potato uses and exports

Potatoes are grown commercially in 36 of the 50 states in the US. Total US production in 2010 was 40.4 billion lbs, with total value of $3.72 billion, planted on 1.03 million acres (NPC 2012). The commercial uses include 37% as frozen, 27% sold fresh, 14% chips, 8% dehydrated, 5% seed potatoes, and 1% canned (NPC 2012). Annual per capita consumption in the US was expected to be approximately 112 lbs per person in 2012 (NPC 2012).

The states with the largest volume and sales value in 2009 were Idaho, Washington, Wisconsin, Colorado, Oregon, Minnesota, North Dakota, Michigan, Maine, and California (USDA-NASS 2010b). These data represent the combined markets including all processing and fresh varieties.

During the 2009/10 marketing year (September-August), US exports of all potatoes and potato products (including starch) totaled $1.19 billion—essentially unchanged from a year earlier (USDA ERS 2010). Japan remained the top foreign market for U.S. potatoes with 28% of the total export value; primarily frozen french fries, other frozen potato products, potato flakes and granules. Japan was followed by Canada (24 % of export value), Mexico (11 %), South Korea (5 %), and China (4 %).

Potato exports from the U.S. in 2010 totaled 5.7 billion lbs (farm weight of potatoes) and 812 million lbs of fresh potatoes. Most of the processed potato exports were frozen, representing 51.4% of total farm weight production (USDA ERS 2010).

10.3. Submissions to Other Regulatory Agencies

Approval for these events will be sought in Canada, Japan, Mexico, and the US. These approvals should ensure continued trade with key export markets.

10.4. Impact on the Organic Market

Organic potatoes in 2008 represented less than 1.0% (0.7%) of total potato production with 8,273 acres of certified organic potatoes (USDA ERS 2011). Of the 8,273 organic acres, California, Washington, and Oregon were the top three organic potato producers with 3,697 acres, 1,600 acres, and 1,101 acres, respectively. Potato varietal selection and breeding are similar between organic and conventional potato production as commercial potatoes are predominantly planted from tuber seed. Risks to organic growers would be most likely to occur with accidental mixing of planting material or of potatoes in farming, transportation, or processing channels. These risks are the same as those that organic growers already experience when keeping their organically grown potatoes separate from conventionally grown potatoes. Because potatoes are clonally propagated, the risk of contaminating seed supplies through cross-pollination is negligible. Organic farmers routinely plant organic tuber seed material and any incidence of cross-pollination in production fields will not affect the harvested potatoes. Producers of organic true potato seed will be able to protect their seed from Innate™ material by ensuring that the seed production fields are sufficiently isolated from Innate™ potato fields. Potential outcrossing can be prevented by separating fields planted for organic TPS production by at least 20 meters from fields planted with Innate™ potatoes (Conner and Dale 1996).

10.5. History of Biotech Potatoes

A review of the history of the introduction of genetically modified potatoes in 1995 in the U.S. (Thornton 2008) indicates the importance of a careful assessment of the performance of Innate™ events, synchronizing approvals in North American and trading countries, and weathering public acceptance issues that may result from activism against food industry members that adopt the new technology.

This history points to the importance of consumer acceptance when introducing any Innate™ crops and products to the market. Since the time when biotech potatoes were on the market briefly, the adoption of biotechnology in the food supply has increased consistently. Herbicide tolerant soybeans have increased from 54% to 93% of the U.S. soybean acreage (USDA-NASS 2010a) since 2000. Genetically modified (GM) corn production has increased from 25% of U.S. corn acreage in 2000 to 86 % of corn acreage in 2010 (USDA-NASS 2010a). These two crops provide significant quantities of GM-derived ingredients in widely marketed processed food and feed products, such as protein, oils, starches, and sweeteners. Many of the conventional processed potato products made from the events presented in this petition already contain one or more of these GM ingredients. Importantly, the low acrylamide potato events contain a new trait that has value to the consumer. The growing interest in improving food safety by reducing acrylamide in processed potatoes is expected to provide an incentive for enhanced consumer acceptance of these new events and the products derived from them.

10.6. Stewardship of Innate™ Potatoes

Stewardship of Potato Events

Throughout development of the Innate™ potatoes, the Biotechnology Quality Management System (USDA-BRS Compliance assistance program) has served as a stewardship program to conduct field trials, monitor movement, and complete testing covering the duration of the regulated status of Innate™ potatoes. As a part of BQMS, qualified and experienced potato growers were identified, and agreements were established to ensure compliance and conformance requirements for growing Innate™ potatoes. Processes were developed that included Standard Operating Procedures (SOPs) and forms for all critical control points to guide internal and external collaborators to conduct activities using Innate™ potatoes.

The following management procedures were included for effective implementation of BQMS:

1. Document and record control
2. Resource management
3. Training external and internal associates
4. Planning and process realization
5. Conducting internal audits
6. Implement corrective / preventive actions
7. Continuous improvement of the system
8. Resolution of compliance issues

Targeted training programs were used to educate and bring awareness on handling Innate™ potatoes in storage, transport, planting, harvest, post-harvest, processing, and final disposition to ensure no Innate™ potato material entered into the food or feed channels prior to de-regulation. Both internal and external audits were used to verify that planned activities occurred and personnel were adhering to the protocols recommended by BQMS. This stewardship program includes corrective and preventive actions

to avoid compliance and conformance incidents. While BQMS served as a quality management system during product development, we anticipate a full extension of such methods as outlined in the 'Excellence Through Stewardship®' (BIO 2007) program.

Identity Preservation
To help prevent the trade disruptions experienced with other potato events, international approvals will be pursued from key trading partner countries before the Innate™ varieties are launched commercially. The initial Innate™ introduction will build up slowly as seed becomes available and will be controlled within existing processing channels to ensure that potatoes enter only the intended markets. This will provide an extended period of time to assess consumer acceptance and to address grower and industry awareness. A limited introduction in a vertically integrated supply chain will be well controlled by grower and processor agreements. In this situation, conventional products will be considered "identity preserved" with respect to the well-controlled stewardship of the Innate™ crop and its products. As Innate™ potato adoption increases, programs for identity preservation will be implemented as needed. It's expected that development and implementation of identity preservation systems will add some cost to the supply chain. The total costs will depend upon the type and extent of market penetration.

Synchronized Regulatory Approvals
We intend to follow the recommended stewardship policy statement released by the Biotechnology Industry Organization in May 2007.

"To help ensure the continued adoption of agricultural biotechnology globally and to continue to have products of agricultural biotechnology bring value to the marketplace, BIO's Food and Agriculture Section supports actions that facilitate the flow of goods in commerce and minimize trade disruptions. BIO's Food and Agriculture Section believes that henceforth individual member companies should, prior to commercialization meet applicable regulatory requirements in key countries identified in a market and trade assessment that have functioning regulatory systems and are likely to import the new biotechnology-derived plant products."

The voluntary guideline was adopted because, according to BIO: "asynchronous authorizations combined with importing countries maintaining "zero tolerance" for recombinant-DNA products not yet authorized results in the potential for major trade disruptions. The potential occurrences of trade disruptions will only increase given the substantial amount of research that will bring many new products and combinations of products to market."

The submission for nonregulated status in the US will be followed by applications submitted in Canada, Mexico, and Japan, with the intention of completing approvals before crop introduction.

11. Conclusions: Determination of Nonregulated Status for Innate™ Potatoes

Simplot's Innate™ technologies allow us to transform plants with potato DNA and only introducing non-coding DNA (DNA not coding for RNA that is translated into protein) into the plant's genome. We transformed five different varieties with pSIM1278, and selected ten of these events for determination of nonregulated status: F10 and F37 for Ranger Russet, E12, and E24 for Russet Burbank, J3, J55, and J78 for Atlantic, G11 for variety G, and H37 and H50 for variety H. As demonstrated in the data presented in this Petition, the transformed cultivated potato plants are not parasitic, and our data prove there is no reason to believe they are plant pests. Furthermore, the expression of the integrated genetic elements results in traits highly sought-after in the potato industry, namely: reduced black spot bruise, low

asparagine, and lower levels of reducing sugars. Ultimately, we demonstrate that the Innate™ tubers exhibited the desired traits which can lead to lower acrylamide levels in cooked potatoes and, importantly, we showed that the transformed tubers were otherwise substantially equivalent to untransformed controls.

To conclude, we have created plants utilizing biotechnology that are not plant pests and which have resulted from the addition of well characterized non-coding regions from potato or wild potato that are as safe as untransformed potatoes. We therefore seek nonregulated status of potato plants transformed using our Innate™ technologies and submit the evidence that these plants should not be classified as "Regulated articles" as defined under 7 CFR 340.

12. Statement of Grounds Unfavorable

JR Simplot is not aware of any information indicating that Innate™ potatoes may pose a greater plant pest risk than conventional potatoes. There are no adverse environmental consequences anticipated with its introduction based on the data collected to date. The benefits of introduction of commercial events are provided in this submitted petition.

13. Certification

The undersigned certifies that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioners which are unfavorable to the petition.

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14. Record of Field Test Reports

The J.R. Simplot Company has adhered to USDA compliance regulations by submitting the field test reports for each notification (**Table 13**). Volunteer monitoring for 2009, 2010, and 2011 field trials is discussed in **Appendix 10** (**Post Harvest Monitoring**).

Table 13. Field release notifications

USDA Reference Number	Field Trial Year	Effective Start and Expiration Date	Release Sites (by State) Covered by Notification	Field Test Report Submitted (Yes or No)
08-353-103n	2009	1/30/2009 – 1/30/2010	FL	Yes
08-353-104n	2009	4/01/2009 – 4/01/2010	MI	Yes
09-049-114n	2009	4/13/2009 – 4/13/2010	ID, WI	Yes
09-077-112n	2009	4/23/2009 – 4/23/2010	NE	Yes
09-336-103n	2010	1/25/2010 – 7/31/2011	FL	Yes
10-053-132n	2010	4/05/2010 – 4/05/2011	ID, MI, ND, NE, WA, WI	Yes
10-076-103n	2010	4/26/2010 – 4/26/2011	WI	Yes
10-326-103n	2011	1/03/2011 – 1/03/2012	FL	Yes
11-063-103n	2011	4/04/2011 – 4/03/2012	ID, IN, MI, ND, NE, WA, WI	Yes
11-094-106n	2011	5/02/2011 – 5/2/2012	WI	Yes
11-150-101n	2011	6/06/2011 – 6/62012	MI	Yes
11-083-111n	2011	4/11/2011 – 4/11/2012	ID	Yes
11-074-109n	2011	4/04/2011 – 4/04/2012	WA	Yes
12-018-110n	2012	2/01/2012 – 2/01/2013	ID	No

15. Literature Cited

Astwood J, Alibhai M, Lee T, Fuchs R, Sampson H (2000) Identification and characterization of IgE binding epitopes of patatin, a major food allergen of potato. Program and abstracts of the 56th annual meeting of the American Academy of Allergy, Asthma and Immunology, March 3-8, San Diego, Calif. Abstracts 555.

Bamberg JB, del Rio A, Huaman Z, Vega S, Martin M, Salas A, Pavek J, Kiru S, Fernandez C, Spooner D (2003) A decade of collecting and research on wild potatoes of the southwest. Am J of Potato Res, 80: 159-172.

Bamberg JB, del Rio A (2011a) Use of native potatoes for research and breeding. HortScience, 46: 1444-1445.

Bamberg JB, del Rio A (2011b) Diversity relationships in tetraploid wild potato native to the USA [abstract]. Am J of Potato Res, 88:29. http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=252056

Bartlett JG, Alves SC, Smedley M, Snape JW, Harwood WA (2008) High-throughput *Agrobacterium*-mediated barley transformation. Plant Methods, 4: 22.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B (Methodological), 57: 289–300.

BIO (2007) US Biotechnology Industry Organization Product Launch Stewardship Policy. http://www.bio.org/foodag/stewardship/20070521.asp (Accessed 22 May 2011).

CFIA (1996) Biology Document BIO1996-09: The Biology of *Solanum tuberosum* (L.) (Potatoes). Canadian Food Inspection Agency. http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/solanum-tuberosum-l-/eng/1330982063974/1330982145930 (Accessed 23 August 2012).

Chawla R, Shakya R, Rommens CM (2012) Tuber specific silencing of asparagine synthetase-1 reduces the acrylamide-forming potential of potatoes grown in the field without affecting tuber shape and yield. Plant Biotechnol J, 10: 913-924.

Cheng M, Fry JE, Pang SZ, Zhou HP, Hironaka CM, Duncan DR, Conner TW, Wan YC (1997) Genetic transformation of wheat mediated by *Agrobacterium* tumefaciens. Plant Physiol, 115: 971–980.

Chiou TJ, Aung K, Lin SI, Wu CC, Chiang SF, Su CI (2006) Regulation of phosphate homeostasis by microRNA in Arabidopsis. Plant Cell, 18: 412-421.

Conner AJ, Dale PJ (1996) Reconsideration of pollen dispersal data from field trials of transgenic potatoes. Theor Appl Genet, 92: 505-508.

Conner AJ, Mlynárová L, Stiekema WJ, Nap JP (1998) Meiotic stability of transgene expression is unaffected by flanking matrix-associated regions. Mol Breeding, 4: 47-58.

Courvalin P, Weisblum B, Davies J (1977) Aminoglycoside-modifying enzyme of an antibiotic-producing bacterium acts as a determinant of antibiotic resistance in Escherichia coli. P Natl Acad Sci USA, 74: 999-1003.

Daxinger L, Hunter B, Sheikh M, Jauvion V, Gasciolli V, Vaucheret H, Matzke M, Furner, I (2008) Unexpected silencing effects from T_DNA tags in Arabidopsis. Trends Plant Sci, 13: 4-6.

De Buck S, Jacobs A, Van Montagu M, Depicker A (1999) The DNA sequences of T-DNA junctions suggest that complex T-DNA loci are formed by a recombination process resembling T-DNA integration. Plant J, 20: 295-304.

De Buck S, Podevin N, Nolf J, Jacobs A, Depicker A (2009) The T-DNA integration pattern in Arabidopsis transformants is highly determined by the transformed target cell. Plant J, 60: 134-145.

De Neve M, De Buck S, Jacobs A, Van Montagu M, Depicker A (1997) T-DNA integration patterns in co-transformed plant cells suggest that T-DNA repeats originate from ligation of separate T-DNAs. Plant J, 11: 15–29.

De Swert LF, Cadot P, Ceuppens JL (2002) Allergy to cooked white potatoes in infants and young children: A cause of severe, chronic allergic disease. J Allergy Clin Immun, 110: 524-535.

De Swert LF, Cadot P, Ceuppens JL (2007) Diagnosis and natural course of allergy to cooked potatoes in children. Allergy, 62: 750-757.

Dwelle, RB (2003) Potato growth and development. In: Potato Production Systems (Eds. Stark JC and Love S) University of Idaho Agricultural Communications, Moscow, pp. 9-19.

Federal Register (2009) “Acrylamide in Food, Request for Comments and for Scientific Data and Information.” *Federal Register* 74 (26 August 2009): 43134-43138. Print.

Federal Register (2011) ”Pesticides; Data Requirements for Plant-incorporated Protectants (PIPs) and Certain Exemptions for PIPs; Notification to the Secretaries of Agriculture and Health and Human Services, Proposed Rule.” *Federal Register* 76 (16 March 2011): 14358-14359. Print.

Garbarino JE, Belknap WR (1994) Isolation of a ubiquitin-ribosomal protein gene (ubi3) from potato and expression of its promoter in transgenic plants. Plant Mol Biol, 24: 119-127.

Gelvin SB (2003) *Agrobacterium*-mediated plant transformation: the biology behind the "gene-jockeying" tool. Microbiol. Mol Biol R, 67: 16-37.

Gheysen G, Villarroel R, Van Montagu M (1991) Illegitimate recombination in plants: a model for T-DNA integration. Gene Dev, 5: 287-297.

Gutiérrez RA, Stokes TL, Thum K, Xu X, Obertello M, Katari MS, Tanurdzic M, Dean A, Nero DC, McClung CR, Coruzzi GM (2008) Systems approach identifies an organic nitrogen-responsive gene

network that is regulated by the master clock control gene CCA1. P Natl Acad Sci, 105: 4939-4944.

Hagio M, Sakurai I, Sato S, Kato T, Tabata S, Wada H (2002) Phosphatidyglycerol is essential for the development of thylakoid membranes in Arabidopsis thaliana. Plant Cell Physiol, 43: 1456-1464.

Hakimi SM, Krohn BM, Stark DM: Monsanto Technology LLC (2006) United States Patent: Method of imparting disease resistance to plants by reducing polyphenol oxidase activities. Patent No.: US 7,122,719 B2.

Hall KD, Holloway RL, Smith DT (2000) Texas Crop Profile: Potatoes. Texas Agricultural Extension Service, Texas A & M University, TX.

Hanneman RE (1994) The testing and release of transgenic potatoes in the North American Center of diversity. In: Biosafety of Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere (Eds. Krattiger AF and Rosemarin A). ISAAA, Ithaca and SEI, Stockholm, pp. 47-67.

Hijmans RJ, Spooner DM (2001) Geographic distribution of wild potato species. Am J of Botany, 88: 2101-2112.

Hokanson K, Heron D, Gupta S, Koehler S, Roseland C, Shantharam S, Turner J, White J, Schechtman M, McCammon S, Bech R (1999) The concept of familiarity and pest resistant plants. Proceedings of a workshop on: Ecological Effects of pest resistance genes in managed ecosystems. January 31-February 3, 1999, Bethesda, MD. USDA-APHIS Plant Protection and Quarantine, Scientific Services.

Hopkins BG, Stark J, Hafez SL, Hutchinson PJS, Patterson P, Miller J, Thornton M, and Alvarez, J (2003) Field Selection, Crop Rotations, and Soil Management. In: Potato Production Systems (Eds. Stark JC and Love S) University of Idaho Agriculutral Communications, Moscow, pp. 1-7.

IPD (2011) Inter-genebank Potato Database. Wild Potato Collection. http://research.cip.cgiar.org/IPD/ (Accessed 10 November 2011).

Kim SI, Veena, Gelvin SB (2007) Genome-wide analysis of *Agrobacterium* T-DNA integration sites in the Arabidopsis genome generated under non-selective conditions. Plant J, 51: 779-791.

Kononov ME, Bassuner B, Gelvin SB (1997) Integration of T-DNA binary vector 'backbone' sequences into the tobacco genome: evidence for multiple complex patterns of integration. Plant J, 11: 945-957.

Kozukue N, Yoon KS, Byun GI, Misoo S, Levin CE, Friedman M (2008) Distribution of glycoalkaloids in potato tubers of 59 accessions of two wild and five cultivated Solanum species. J Agric Food Chem, 56: 11920-11928.

Lazo GR, Stein PA, Ludwig RA (1991) A DNA transformation-competent Arabidopsis genomic library in *Agrobacterium*. Biotechnology, 9: 963-967.

Lechtenberg B, Schubert D, Forsbach A, Gils M, Schmidt R (2003) Neither inverted repeat T-DNA configurations nor arrangements of tandemly repeated transgenes are sufficient to trigger transgene silencing. Plant J, 34: 507-517.

Li L, Steffens JC (2002) Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. Planta, 215: 239-247.

Llorente B, Alonso GD, Bravo-Almonacid F, Rodríguez V, López MG, Carrari F, Torres HN, Flawiá MM (2011) Safety assessment of nonbrowning potatoes: opening the discussion about the relevance of substantial equivalence on next generation biotech crops. Plant Biotechnol J, 9: 136-150.

Lorberth R, Ritte G, Willmitzer L, Kossmann J (1998) Inhibition of a starch-granule-bound protein leads to modified starch and repression of cold sweetening. Nat Biotechnol, 16: 473-477.

Love S (1994) Ecological risk of growing transgenic potatoes in the United States and Canada. Am Potato J, 71: 647-658.

Lusk JL, Rozan A (2006) Consumer acceptance of ingenic foods. Biotechnol J, 1: 1433-1434.

Lusk JL, Sullivan P (2002) Consumer acceptance of genetically modified foods. Food Technology, 56: 32-37.

Masclaux-Daubresse C, Reisdorf-Cren M, Pageau K, Lelandais M, Grandjean O, Kronenberger J, Valadier MH, Feraud M, Jouglet T, Suzuki A (2006) Glutamine synthetase- glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink-source nitrogen cycle in tobacco. Plant Physiol, 140: 444-456.

Mayer AM (2006) Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry, 67: 2318-2331.

McGrath RB, Coruzzi GM (1991) A gene network controlling glutamine and asparagines biosynthesis in plants. The Plant J, 1: 275-280.

Mignery GA, Pikaard CS, Park WD (1988) Molecular characterization of the patatin multigene family of potato. Gene, 62: 27-44.

Müller AJ, Mendel RR, Schiemann J, Simoens C, Inzé D (1987) High meiotic stability of a foreign gene introduced into tobacco by *Agrobacterium*-mediated transformation. Mol Gen Genet, 207: 171-175.

Nakata PA, Anderson JM, Okita TW (1994) Structure and expression of the potato ADP-glucose pyrophosphorylase small subunit. J Bio Chem 269: 30798-30807.

National Atlas (2004a) "Colorado." *The National Atlas of the United States of America*. 2004. http://www.nationalatlas.gov (Accessed 11 November 2011).

National Atlas (2004b) "Texas." *The National Atlas of the United States of America*. 2004. http://www.nationalatlas.gov (Accessed 12 November 2011).

NPC (2012) National Potato Council, 2012 Potato Statistical Yearbook.

NTP (2012) Technical Report on the Toxicology and Carcinogenesis Studies of Acrylamide in F344/N Rats and B6C3F1 Mice. National Toxicology Program. National Institutes of Health. Public Health Service. U.S. Dept. of Health and Human Services.

Obrien J, Morrissey PA (1989) Nutritional and toxicological aspects of the Maillard browning reaction in foods. Crit Rev Food Sci, 38: 211-248.

OECD (1997) Organization for Economic Co-Operation and Development, Consensus document on the biology of *Solanum tuberosum* subsp*. tuberosum* (potato), 38 pages.

OEHHA (2011a) Acrylamide: frequently asked questions. Office of Environmental Health Hazard Assessment. http://www.oehha.ca.gov/prop65/acrylamideqaall.pdf (Accessed 24 May 2011).

OEHHA (2011b) Proposition 65: Acrylamide (2011). Office of Environmental Health Hazard Assessment. http://www.oehha.ca.gov/prop65/acrylamide.html (Accessed 24 May 2011).

Oltmanns H, Frame B, Lee LY, Johnson S, Li B, Wang K, Gelvin SB (2010) Generation of backbone-free, low transgene copy plants by launching T-DNA from the *Agrobacterium* chromosome. Plant Physiol, 152: 1158-1166.

Pallais N, Mulcahy D, Fong N, Falcon R, Schmiediche P (1988) The relationship between potato pollen and true seed: effects of high temperature and pollen size. In Sexual Reproduction in Higher Plants. Spinger-Verlag, Berlin Heidelberg.

Percival G (1999) Light-induced glycoalkaloid accumulation of potato tubers (*Solanum tuberosum L*). J Sci Food Agric, 79: 1305-1310.

Plaisted RL (1980) Potato. In: Hybridization of crop plants (Eds. Fehr, W.R. and Hadley, H.H.). American Society of Agronomy and Crop Science Society of America, publishers, Madison, Wisconsin, 483-494.

Prescott EM, Proudfoot NJ (2002) Transcriptional collision between convergent genes in budding yeast. P Natl Acad Sci USA, 99: 8796-8801.

Ramon M, Rolland F, Sheen J (2008) Sugar Sensing and Signaling. The Arabidopsis Book (TAB), ISSN: 1543-8120 http://www.aspb.org/publications/arabidopsis/pages 1-22.

Richael CM, Kalyaeva M, Chretien RC, Yan H, Adimulam S, Stivison A, Weeks JT, Rommens CM (2008) Cytokinin vectors mediate marker-free and backbone-free plant transformation. Transgenic Res, 17: 905-917.

Sallaud C, Meynard D, van Boxtel J, Gay C, Bès M, Brizard JP, Larmande P, Ortega D, Raynal M, Portefaix M, Ouwerkerk PB, Rueb S, Delseny M, Guiderdoni E (2003) Highly efficient production and characterization of T-DNA plants for rice (Oryza sativa L.) functional genomics. Theor Appl Genet, 106: 1396-1408.

Sinden SL (1987) Potato glycoalkaloids. Acta Hortic., 207: 41-48.

Skirvin RM, Norton M, McPheeters KD (1993) Somaclonal variation: has it proved useful for plant improvement? Acta Horticulturae, 336: 333-340.

Smigocki AC, Owens LD (1988) Cytokinin gene fused with a strong promoter enhances shoot organogenesis and zeatin levels in transformed plant cells. P Natl Acad Sci USA, 85: 5131-5135.

Spooner DM, van den Berg RG, Rodriguez A, Bamberg JB, Hijmans RJ, Cabrera SIL (2004) Wild potatoes (*Solanum* section *Petota*: *Solanaceae*) of North and Central America. Syst Bot Monogr, 68: 1-209.

Spooner DM, McLean K, Ramsay G, Waugh R, Bryan GJ (2005) A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. P Natl Acad Sci USA, 102: 14694-14699.

Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert MC, Riediker S (2002) Acrylamide from Maillard reaction products. Nature, 419: 449-50.

Steffens JC, Harel E, Hunt MD (1994) Polyphenol Oxidase. In: Genetic Engineering of Plant Secondary Metabolism (Ed. Ellis BE) Plenum Press, New York, pp. 275-312.

Thipyapong P, Hunt MD, Steffens JC (2004) Antisense downregulation of polyphenol oxidase results in enhanced disease susceptibility. Planta, 220: 105-117.

Thipyapong P, Stout MJ, Attajarusit J (2007) Review: Functional analysis of polyphenol oxidases by antisense/sense technology. Molecules, 12: 1569-1595.

Thornton M (2008) The rise and fall of NewLeaf™ potatoes. http://nabc.cals.cornell.edu/pubs/nabc_15/chapters/Thornton.pdf (Accessed 31 May 2011).

Tuteja JH, Zabala G, Varala K, Hudson M, Vodkin LO (2009) Endogenous, tissue-specific short interfering RNAs silence the chalcone synthase gene family in glycine max seed coats. Plant Cell, 21: 3063-3077.

Urquhart AA, Joy KW (1981) Use of phloem exudates technique in the study of amino acid transport in pea plants. Plant Physiol, 68: 750-754.

US EPA (2011) United States Environmental Protection Agency. Introduction to Biotechnology Regulation for Pesticides. http://epa.gov/oppbppd1/biopesticides/regtools/biotech-reg-prod.htm (Accessed 27 January 2012).

USDA ERS (2010) Vegetables and Melons Outlook. United States Department of Agriculture Economic Research Service. http://www.ers.usda.gov/publications/vgs (Accessed 2 April 2012).

USDA ERS (2011) Organic Production. United States Department of Agriculture Economic Research Service. http://www.ers.usda.gov/Data/Organic (Accessed 15 June 2011).

USDA-NASS (2010a) United States Department of Agriculture, National Agricultural Statistics Service. Acreage from June 2000-June 2010 (Accessed 24 May 2011).

USDA-NASS (2010b) United States Department of Agriculture, National Agricultural Statistics Service. Potatoes 2009 Summary ISSN: 1949-1514 (Accessed 24 May 2011).

Valentines MC, Vilaplana R, Torres R, Usall J, Larrigaudiere C (2005) Specific roles of enzymatic browning and lignifications in apple disease resistance. Postharvest Biotech and Tech, 36: 227-234.

Van Haaren MJJ, Sedee NJA, de Boer HA, Schilperoort RA, Hooykaas PJJ (1989) Mutational analysis of the conserved domains of a T-region border repeat of Agrobacterium tumefaciens. Plant Mol Bio, 13: 523-531.

Visser RGF, Stolte A, Jacobsen E (1991) Expression of a chimeric granule-bound starch synthase-GUS gene in transgenic potato plants. Pant Mol Bio, 691-699.

Webb RE, Wilson DR, Shumaker JR, Graves B, Henninger NR, Watts J, Frank JA, Murphy HJ (1978) Atlantic: a new potato variety with high solids, good processing quality, and resistance to pests. Am Potato J, 55: 141-145.

Windels P, DeBuck S, VanBockstaele E, DeLoose M, Depicker A (2003) T-DNA Integration in Arabidopsis Chromosomes. Presence and Origin of Filler DNA Sequences. Plant Physiol, 133: 2061-2068.

Wolters AA, Trindade LM, Jacobsen E, Visser RGF (1998) Fluorescence in situ hybridization on extended DNA fibres as a tool to analyse complex T-DNA loci in potato. Plant J, 13: 837-847.

Yan H, Chretien R, Ye J, Rommens CM (2006) New construct approaches for efficient gene silencing in plants. Plant Physiol, 141: 1508-1518.

Yang L, Ding J, Zhang C, Jia J, Weng H, Liu W, Zhang D (2005) Estimating the copy number of transgenes in transformed rice by real-time quantitative PCR. Plant Cell Rep, 23: 759-763.

Zhu W, Ouyang S, Iovene M, O'Brien K, Vuong H, Jiang J, Buell CR (2008) Analysis of 90 Mb of the potato genome reveals conservation of gene structures and order with tomato but divergence in repetitive sequence composition. BMC Genomics, 9:286.

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Appendix 2

Evidence for the Absence of Vector Backbone DNA

INDEX

INTRODUCTION

Three methods were used to ensure that the events do not contain vector backbone DNA. The first method was based on the presence of the negative selectable isopentenyl isomerase (*ipt*) marker gene in the vector backbone. During transformation, inadvertent transfer of backbone DNA comprising the *ipt* gene expression cassette from *Agrobacterium* to plant cells would trigger *ipt* gene expression and, consequently, the formation of the cytokinin-type hormone isopentenyladenosine. Shoots from transformed *ipt* gene-expressing cells develop a "cytokinin-overproduction" phenotype that causes stunted phenotype, abnormal leaves, and an inability to root. Only shoots that were phenotypically-indistinguishable from controls were selected and allowed to develop into whole plants. This screening step helped to guarantee that transformed plants lack a functionally-active backbone marker gene, but could not detect the presence of other backbone DNA in the events. The second method was used on events that had passed the first screening method. This method confirmed the absence of backbone DNA with Southern blot hybridization. The third method was designed to amplify fragments indicative of junctions between DNA insert border regions and flanking backbone DNA or regions within the backbone DNA that flank the DNA insert.

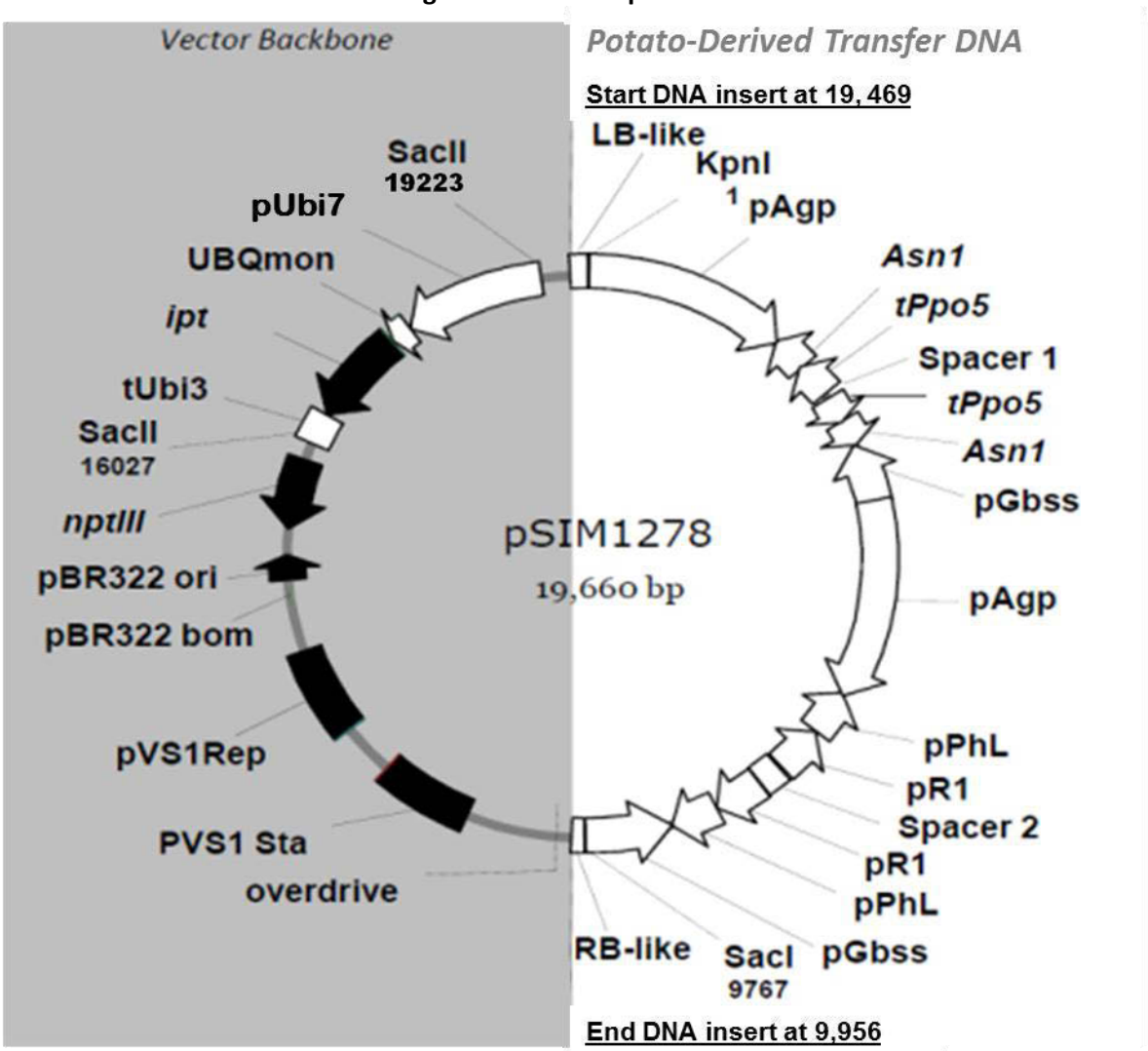
RESULTS

Richael *et al.* (2008) previously showed the efficacy of the *ipt* gene lowers the frequency of transformation events carrying inadvertent vector backbone DNA in addition to the actual DNA insert. Upon transformation, shoots displaying a cytokinin-overproduction phenotype (expressing the *ipt* gene) were simply ignored, and only those indistinguishable from wild-type were transferred and analyzed by PCR for the presence of a DNA insert. Transformed shoots were propagated in tissue culture to generate "events", and three plants of each event were planted in the greenhouse, together with untransformed controls, for molecular analyses.

*Eco*RI-digested DNAs of the 10 events, their untransformed counterparts, and various positive controls were hybridized with a total of 6 probes that span overlapping segments of the vector backbone (**Figure 1 and 2,** below, and **Table 1** and **Figure 15** in **Materials and Methods**). As shown in **Figure 2**, some gaps exist between probes 4 and 5, and probes 5 and 6. The section of vector backbone between probes 4 and 5 contains the terminator for ubiquitin (from *S. tuberosum*); thus the probes were not designed to detect this section that contains potato genomic DNA. The non-overlapping segment between probes 5 and 6 also contains DNA belonging to the Ubi7 promoter and the ubiquitin monomer (UBQmoni), also from potato. The positive controls were obtained by transforming potato with pSIM1278 and selecting for plants containing backbone (G23 contains most of the backbone, and TT4 and T130 carry the entire backbone). In addition to the 10 events considered for deregulation, two events, E67 and G9, were included in most analyses. The lack of bands corresponding to the 6 probes and the 3 positive controls (TT4, T130, and G23) as shown in **Figures 3-10** verify that the 10 events do not contain vector backbone DNA. When evaluating the gels, the presence of any band indicates hybridization with the probes, yet will vary in size from the positive controls, hence they may not line up on the gel.

In some cases, Southern blots shown in this appendix will use the same probes and enzyme digests as found in other studies (for example, see **Appendix 1. Characterization of Inserted DNA**). The observations may differ, depending upon the conditions under which the gels were run. Therefore all bands described in the tables representing Southern blot data in this appendix refer to the gels presented within this appendix.

Figure 1. Vector of pSIM1278



The vector backbone region, on the left, starts at position 9,957-bp and ends at 19,468-bp (= 9,512-bp). The backbone DNA consists mainly of bacterial DNA and is intended only to support maintenance of the DNA insert prior to plant transformation. The DNA insert region, including flanking Border sequences, is on the right from 19,469-bp to 19,660 and from 1-bp to 9,956, which is a total of 10,148-bp. The DNA insert consists of T-DNA border-like sequences and potato genomic DNA which are intended for stable integration into the plant genome.

Figure 2. Probes for the backbone of pSIM1278 (9512 bp)

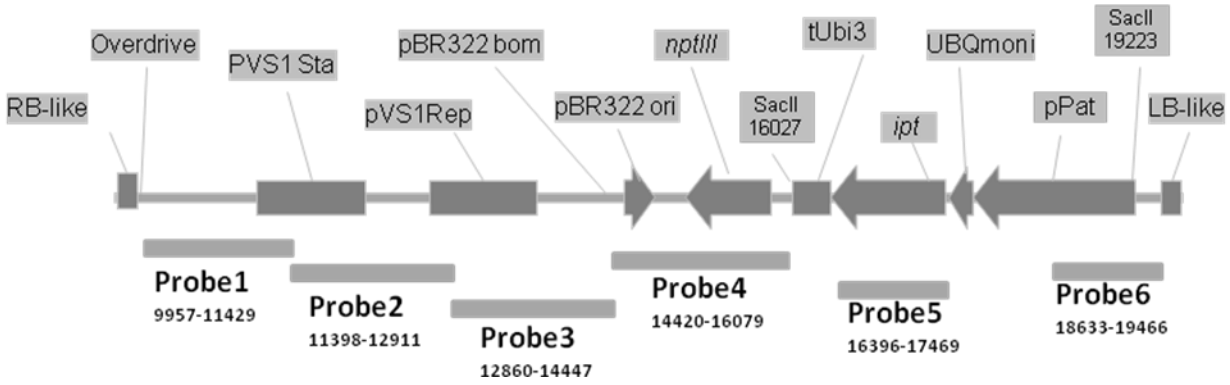


Table 1. Genetic Elements of the pSIM1278 Backbone

Genetic Element	Origin	Intended Function	Other effects on plant	Genbank Accession Number	Start-End Point in pSIM1278 (bp)	Reference
SacII restriction site	*S. tuberosum*	Site for connection of Ubi7 promoter with LB flanking sequence.	*None*	AJ272136.1	19,219-19,224	
Polyubiquitin promoter (**Ubi7**) including the coding sequence for a 76-amino-acid potato ubiquitin monomer (**UBQmon**)	*S. tuberosum* var. Ranger Russet	Drives expression of the *ipt* backbone marker gene	*None*	U26831.1	17,479-19,218	
Isopentenyl transferase (***ipt***) gene	*Agrobacterium tumefaciens*	condensation of AMP and isopentenylpyrophosphate to form isopentenyl-AMP, a cytokinin	*Cytokinin formation*	NC_002377.1	16,744-17,466	Smigocki and Owens 1988
Terminator of the ubiquitin-3 gene (**tUbi3**)	*S. tuberosum*	Terminate *ipt* gene transcription	*None*	GP755544.1	16,038-16,392	Garbarino and Belknap 1994
Neomycin phosphotransferase III (***nptIII***) gene	*E. coli*	Aminoglycoside phosphotransferase	*None*	FJ362602.1	15,048-15,842	Courvalin *et al.* 1977
Origin of replication for pBR322 (**pBR322 ori**)	*E. coli*	Start position for plasmid replication in bacterial cells	*None*	J01784.1	14,477-14,757	*
(**pBR322 bom**)	*E. coli*	pBR322 region for replication in *E. coli*	*None*	J01749.1	14,077-14,337	*
pVS1 replicon (**pVS1Rep**)	*Pseudomonas fluorescens* plasmid pVS1	pVS1 region for replication in *Agrobacterium*	*None*	AJ537514.1 (4,501-5,501)	12,667-13,667	*
pVS1 partitioning protein StaA (**PVS1 Sta**)	*Pseudomonas fluorescens* plasmid pVS1	pVS1 stability	*None*	AJ537514.1 (6,095-7,095)	11,074-12,074	*
overdrive	*Agrobacterium tumefaciens*	Enhances cleavage at the Right Border site	*None*	K00549.1 (103-132)	9,963-9,992	*

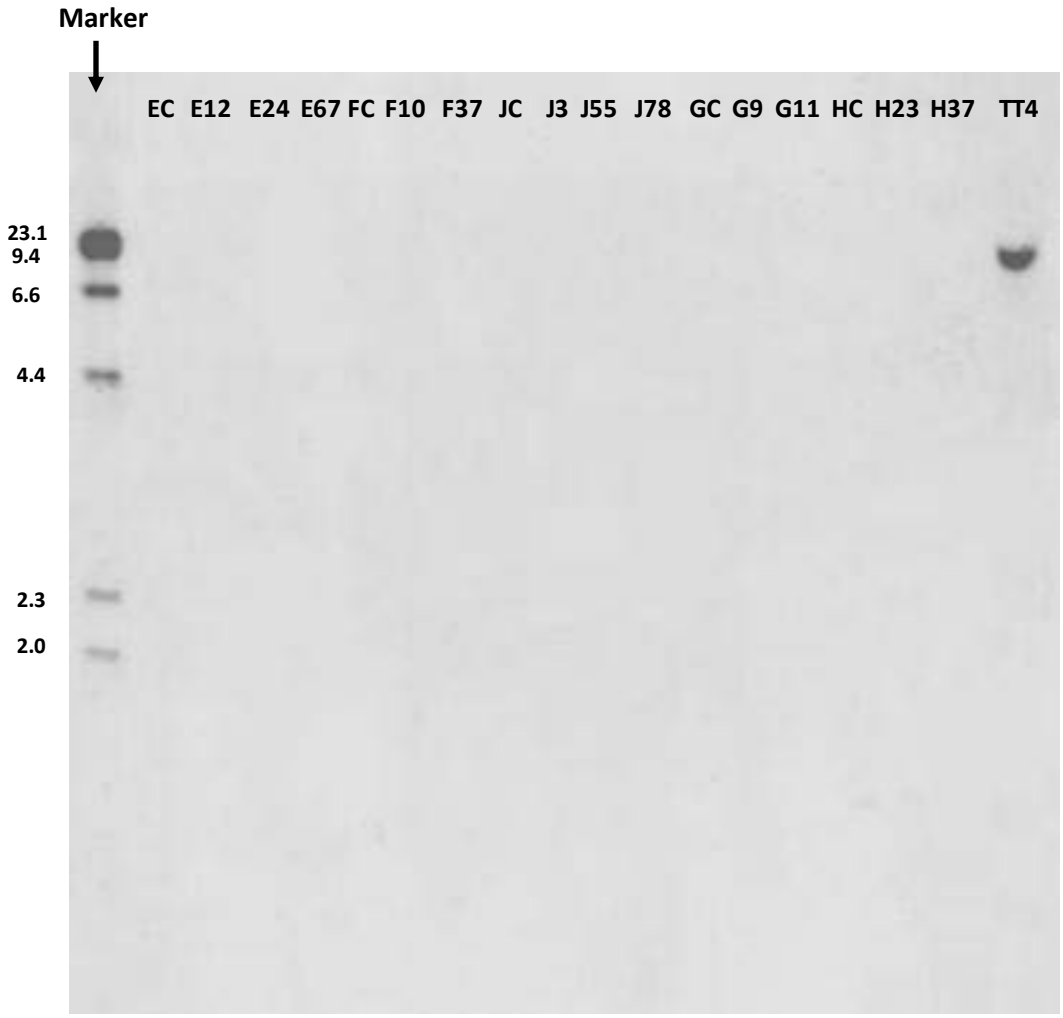
*: see: Cambia (2012) - http://www.cambia.org/daisy/cambia/585.html - (General structure map of pCAMBIA vectors)

For both the Southern analysis and PCR testing, H50 was analyzed separately from the other events. The event H50 was not originally selected for deregulation, but was added later when the event H23 was disqualified for containing backbone. We show the results for all other events (E12, E24, F10, F37, J3, J55, J78, G11, and H37) and probes 1-4 in **Figures 3-6**. None of the untransformed controls nor events showed any band associated with backbone as shown in the lane for TT4. In **Figure 7**, illustrating hybridization with probe 5, we show a band indicating backbone in event H23 (a faint band, different in size from the positive controls), and H23 was then dropped from consideration for deregulation. In **Figure 8**, all lines, including controls, have bands in common with probe 6, indicating that the native promoters have homology with that probe. However, backbone would only be confirmed in an event if there were unique bands associated with the positive controls and not found in the untransformed controls. None of those were observed.

Results for testing all 6 probes with event H50 and its untransformed control are shown in **Figures 9 and 10**. No hybridization occurred with probes 1-5, indicating that no backbone was present (**Figures 9, 10**). Using probe 6, we saw bands in both H50 and its control, again showing homology with that probe and the native promoters. However, there were no unique bands corresponding with positive controls G23 or T130 that would represent backbone (**Figure 10**).

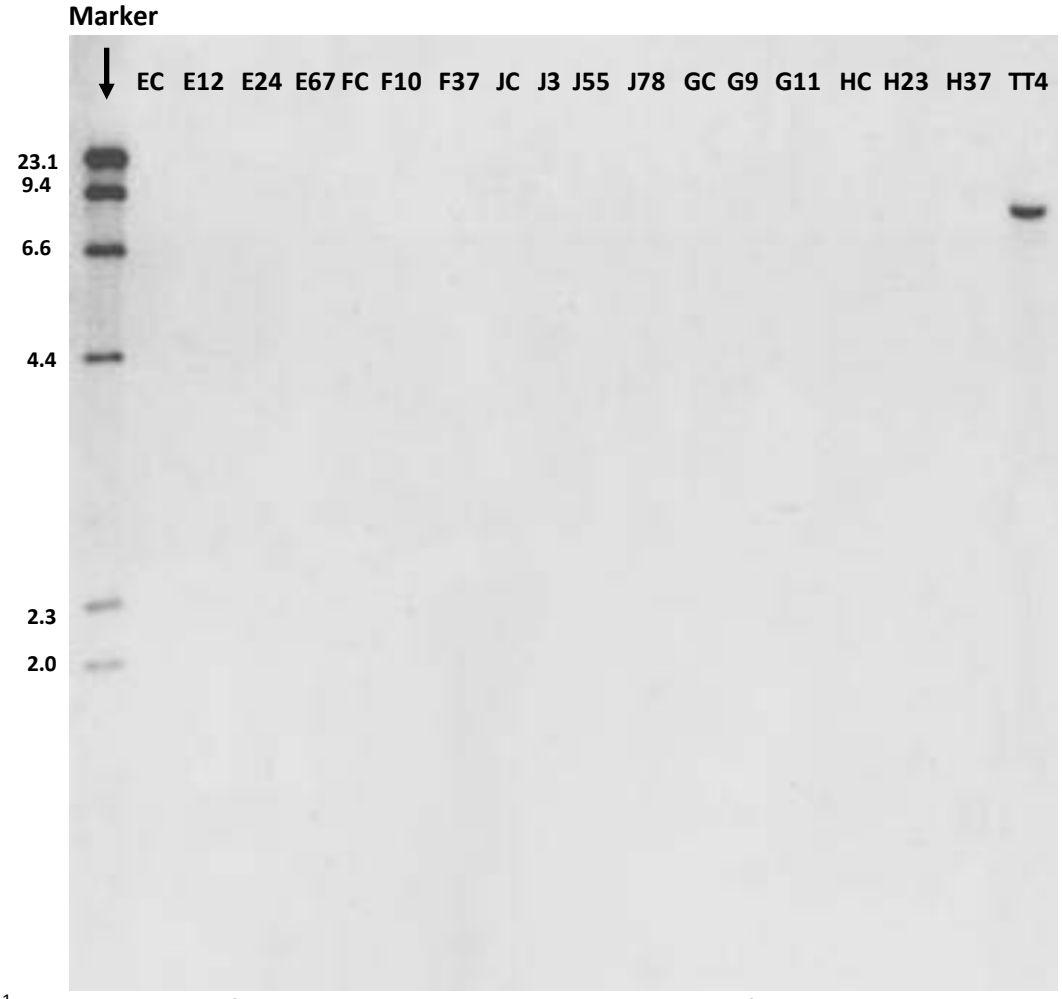
Further confirmation of absence of backbone was obtained using PCR and multiple primers (**Table 3**) associated with the border regions. As shown in **Figures 11 -12**, using multiple primers, no bands were amplified, thus confirming that events and untransformed controls did not contain backbone close to the right border. Additional primers (**Table 3**) were used with PCR and the lack of bands upon amplification confirmed that backbone was not present in the vicinity of the left border (**Figure 13-14**) for all events and the untransformed controls.

Figure 3. DNA Gel Blot Hybridization with Vector Backbone Probe 1[1]



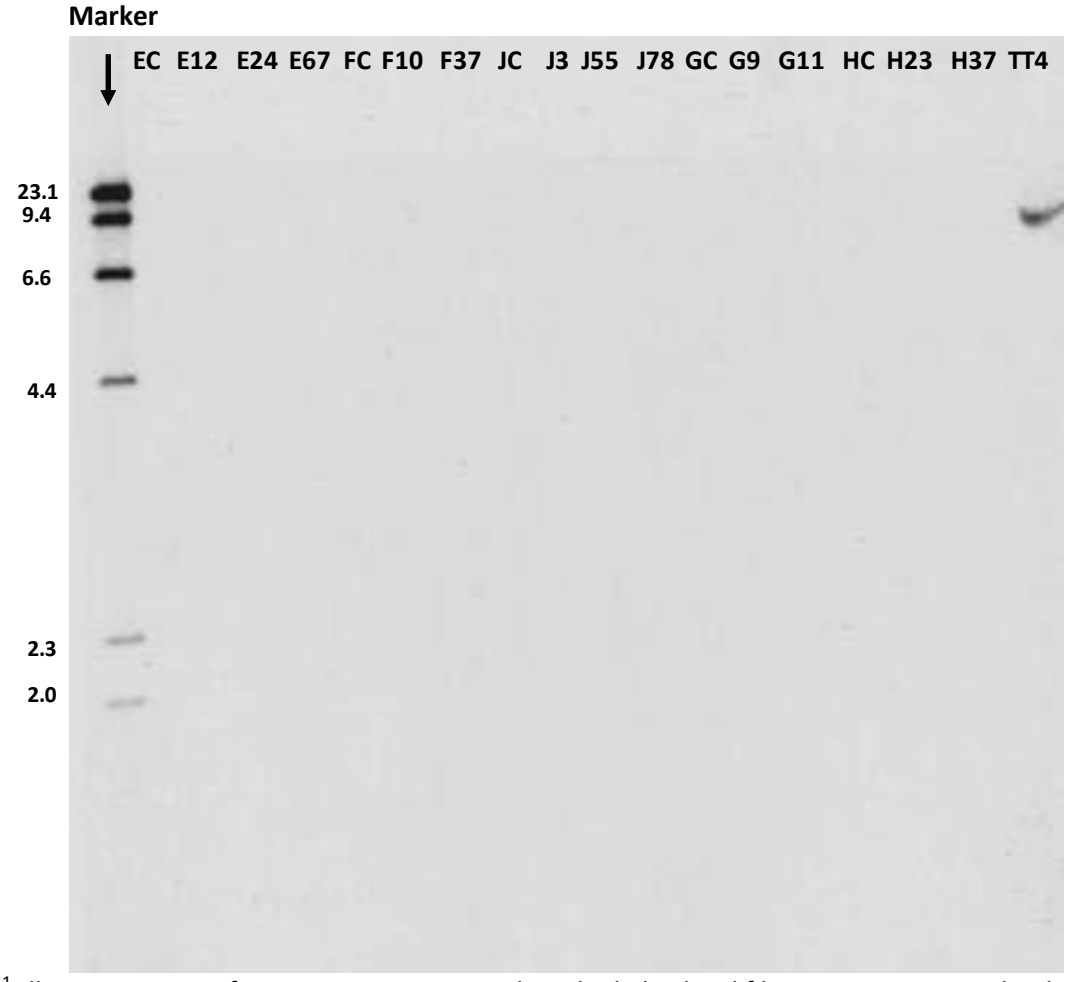
[1]All events, except for H50, are represented on the hybridized filter. EC = Russet Burbank control, FC = Ranger Russet control, JC = Atlantic Control, GC = variety G control, HC = variety H control, TT4 = positive control carrying backbone. Lane 1 = DIG II markers with fragment sizes in kb. E67, G9, and H23 not included in the submission.

Figure 4. DNA Gel Blot Hybridization with Vector Backbone Probe 2[1]



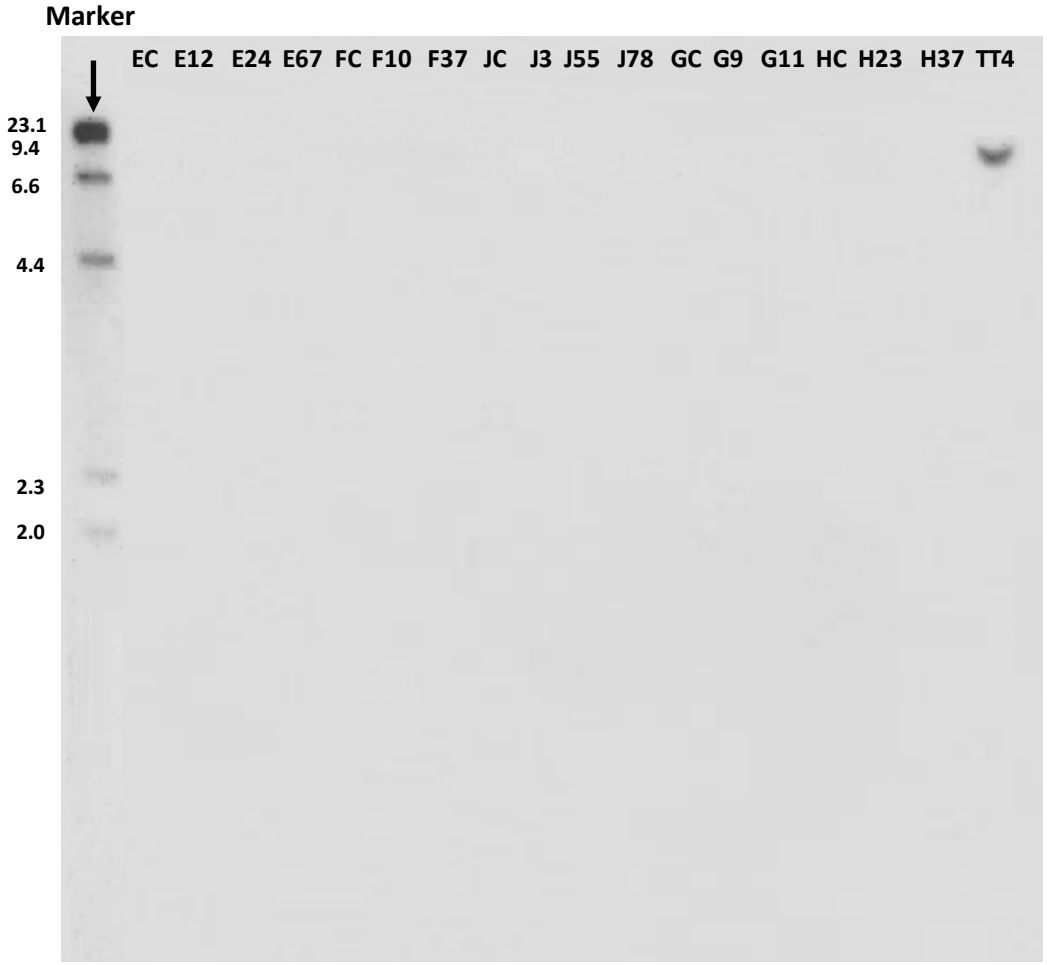
[1]All events, except for H50, are represented on the hybridized filter. EC = Russet Burbank Control, FC = Ranger Russet Control, JC = Atlantic Control, GC = variety G Control, HC = variety H Control, TT4 = positive control carrying backbone. Lane 1 = DIG II markers with fragment sizes in kb. E67, G9, and H23 not included in the submission.

Figure 5. DNA Gel Blot Hybridization with Vector Backbone Probe 3[1]



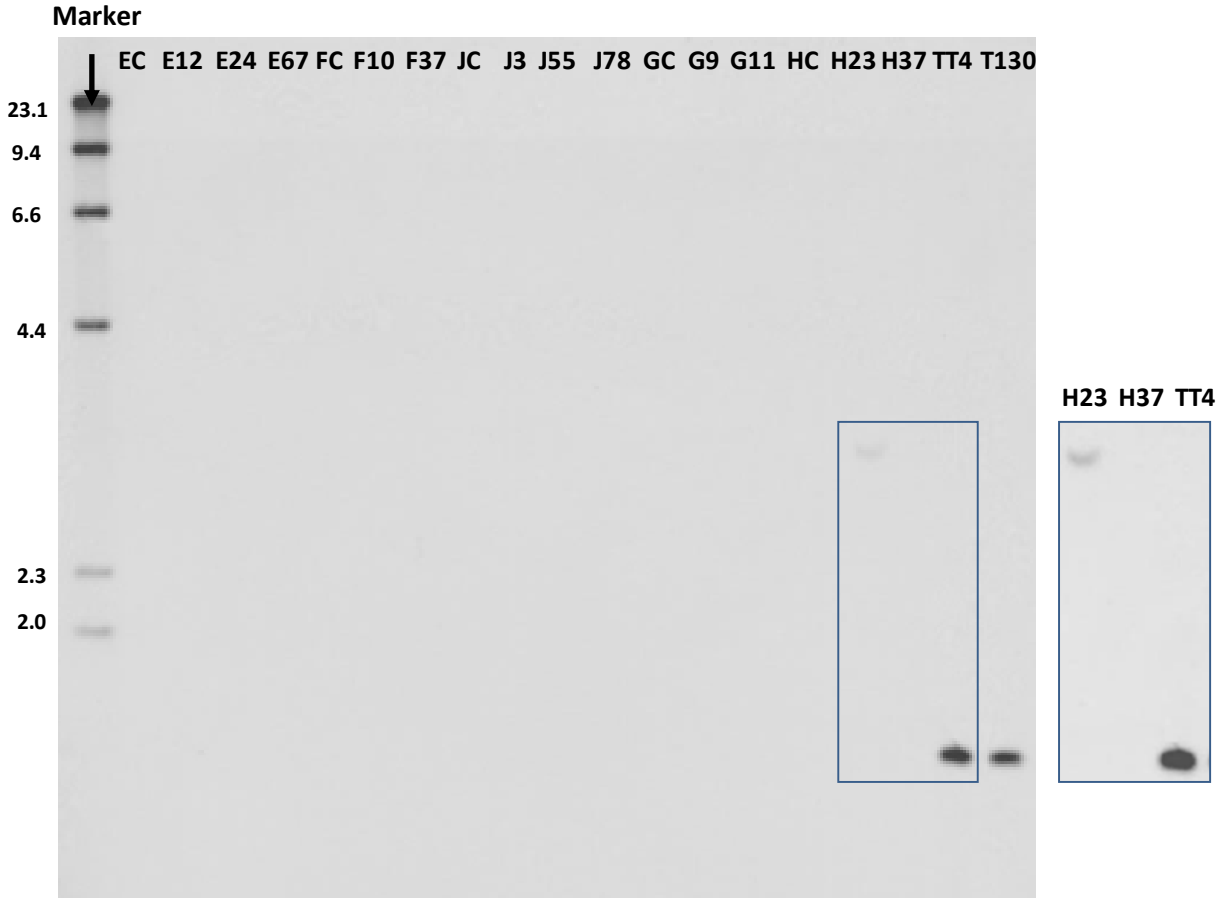
[1]All events, except for H50, are represented on the hybridized filter. EC = Russet Burbank Control, FC = Ranger Russet Control, JC = Atlantic Control, GC = variety G Control, HC = variety H Control, TT4 = positive control carrying backbone. Lane 1 = DIG II markers with fragment sizes in kb. E67, G9, and H23 not included in the submission.

Figure 6. DNA Gel Blot Hybridization with Vector Backbone Probe 4[1]



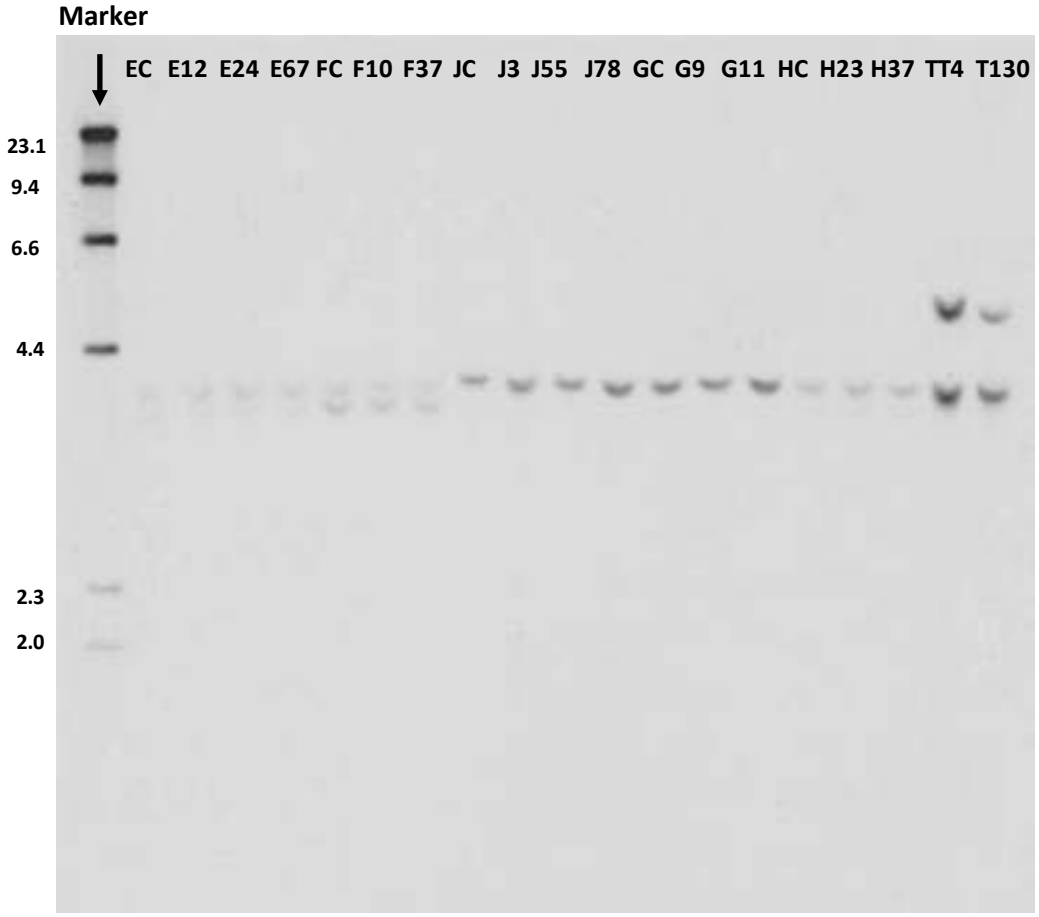
[1]All events, except for H50, are represented on the hybridized filter. EC = Russet Burbank Control, FC = Ranger Russet Control, JC = Atlantic Control, GC = variety G Control, HC = variety H Control, TT4 = positive control carrying backbone. Lane 1 = DIG II markers with fragment sizes in kb. E67, G9, and H23 not included in the submission.

Figure 7. DNA Gel Blot Hybridization with Vector Backbone Probe 5[1]



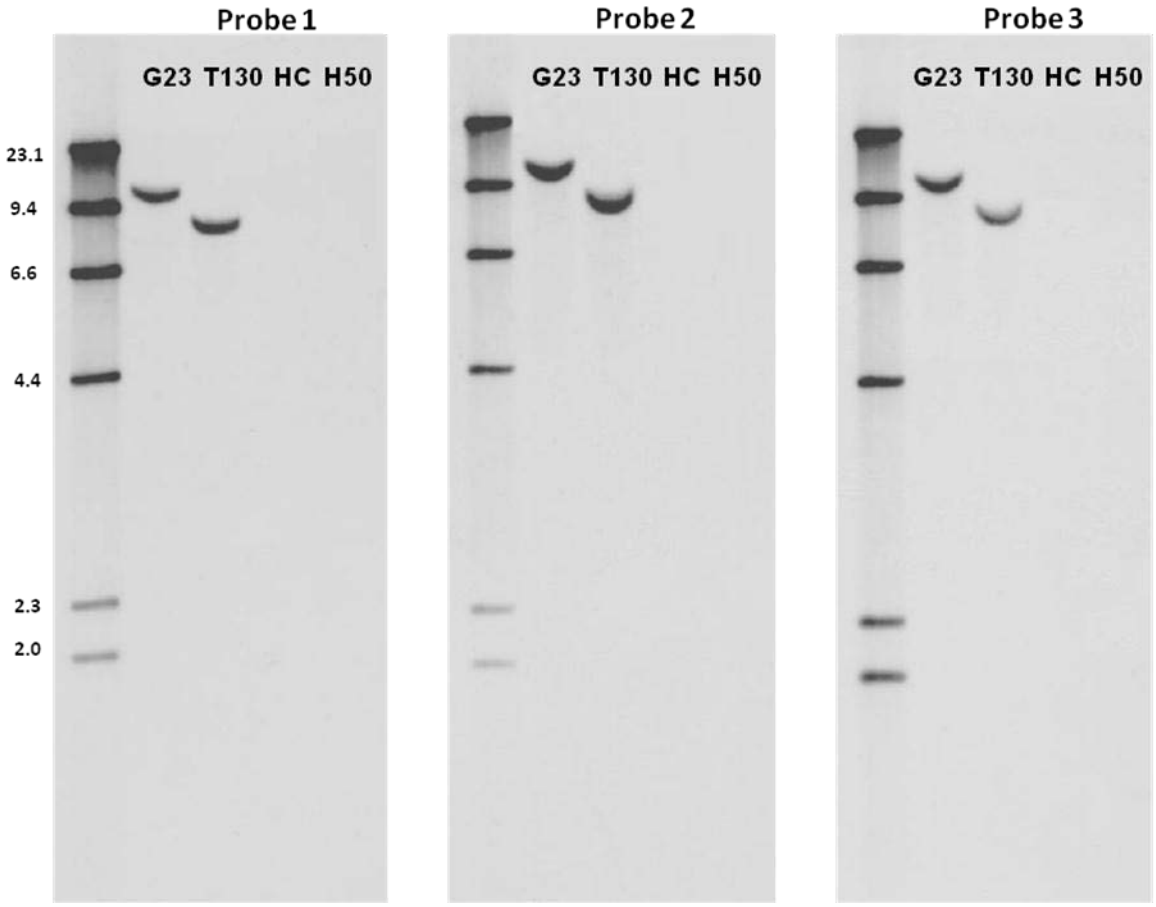
[1]All events, except for H50, are represented on the hybridized filter. EC = Russet Burbank Control, FC = Ranger Russet Control, JC = Atlantic Control, GC = variety G Control, HC = variety H Control, TT4 and T130 are positive controls carrying backbone. The H23 event is not included in this submission as it contains vector DNA. Lane 1 = DIG II markers with fragment sizes in kb. The insert is a section of the filter with a longer exposure time. E67, G9, and H23 not included in the submission.

Figure 8. DNA Gel Blot Hybridization with Vector Backbone Probe 6[1]



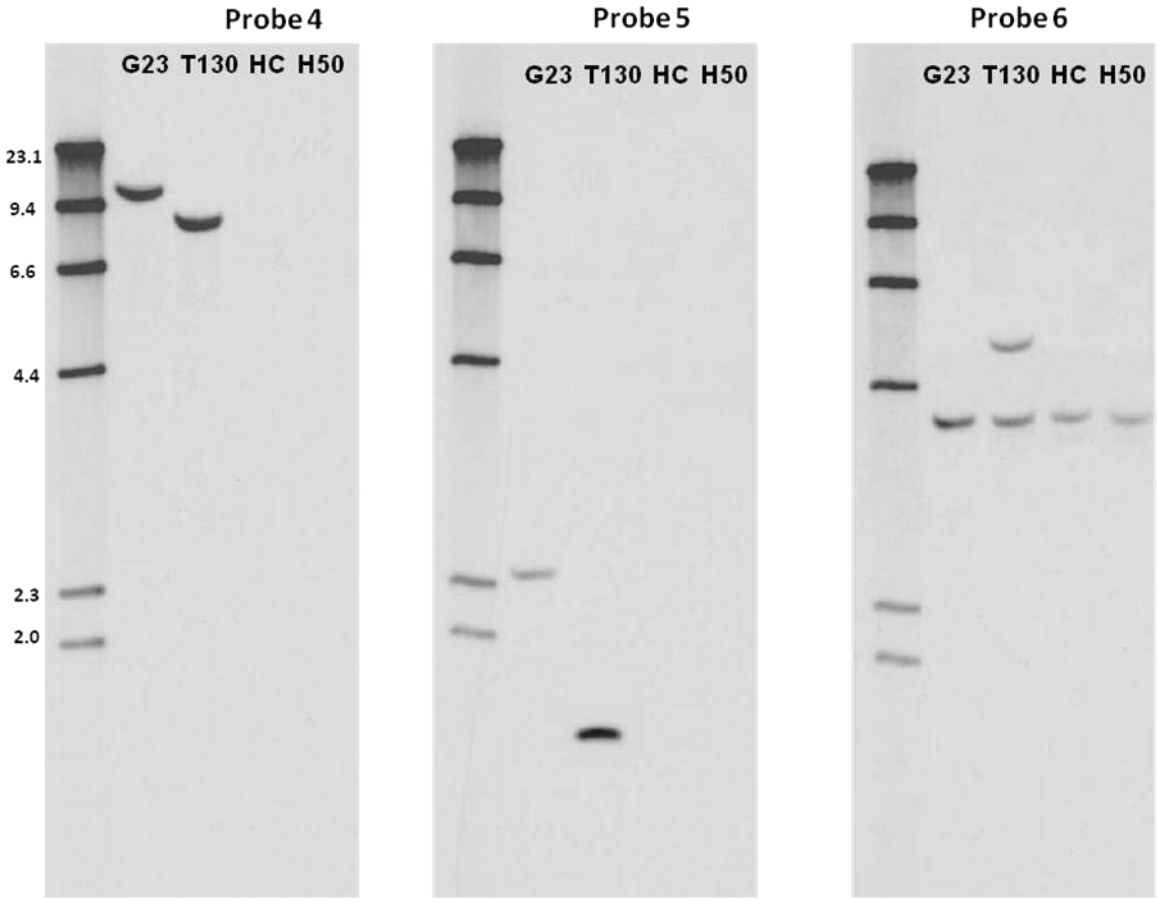
[1]All events, except for H50, are represented on the hybridized filter. EC = Russet Burbank Control, FC = Ranger Russet Control, JC = Atlantic Control, GC = variety G Control, HC = variety H Control, TT4 and T130 are positive controls carrying backbone. Lane 1 = DIG II markers with fragment sizes in kb. The presence of bands common in events and untransformed controls shows that probe 6 has homology with the native promoters. Backbone would be indicated by bands found in the events but not in the untransformed controls; however, none were observed. E67, G9, and H23 not included in the submission.

Figure 9. DNA Gel Blot Hybridization for Event H50 with Vector Backbone Probes 1-3[1]



[1]These membranes carried genomic DNA from positive control events (G23 and T130), negative control (untransformed H, HC) and event H50, hybridized with vector probes 1 to 3. Lane 1 = DIG II markers with fragment sizes in kb.

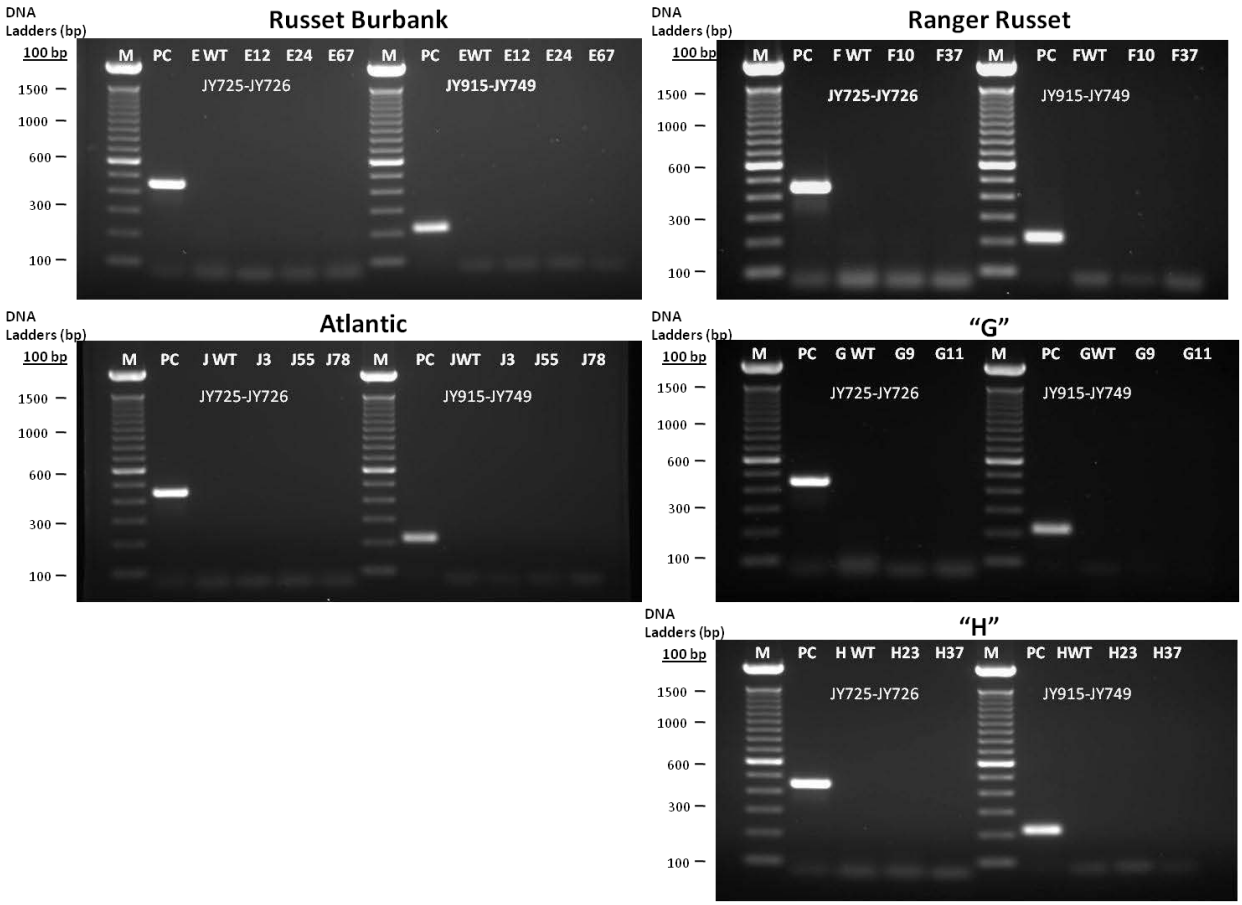
Figure 10. DNA Gel Blot Hybridization for Event H50 with Vector Backbone Probes 4-6[1]



[1]These membranes carried genomic DNA from the positive control lines (G23 and T130), negative control (untransformed H, HC) and event H50, hybridized with vector probes 4 to 6. G23 does not contain the entire backbone; it lacks the DNA sequence associated with probe 6. Lane 1 = DIG II markers with fragment sizes in kb.

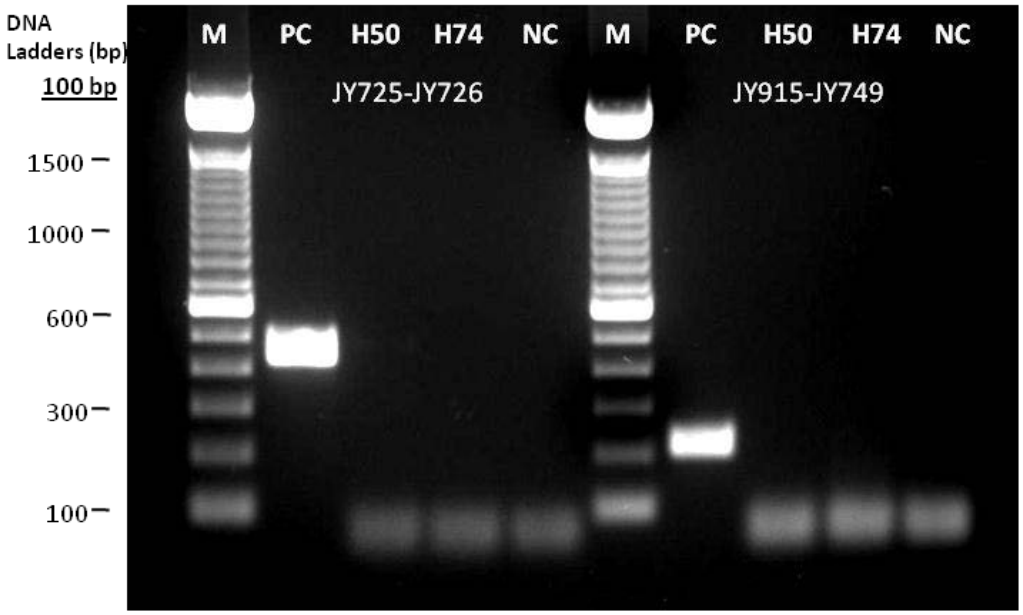
An additional approach to identify vector backbone sequences was based on PCR methods using primers designed to amplify either junctions between the DNA insert border regions and flanking backbone DNA or regions within the backbone DNA that flank the DNA insert (see **Materials and Methods**). Efficacy of the method was confirmed by using pSIM1278 DNA as a positive control. **Figures 11** and **12** show that none of the events presented for deregulation produce PCR bands that are indicative of the presence of vector backbone DNA at the Right Border side of the inserted DNA.

Figure 11. Confirmation of the Absence of Backbone DNA Flanking the Right Border of the DNA insert[1]



[1]Bands on the ethidium bromide-stained gels are indicative for the presence of Right Border/backbone DNA-junctions. Data for all events, except for H50, are shown (see **Figure 12 for H50**). M = 100-bp DNA marker lane (invitrogen), PC = positive plant control, WT = untransformed recipient variety ("wild type", the negative control), primers printed on each gel. E67, G9, and H23 not included in the submission.

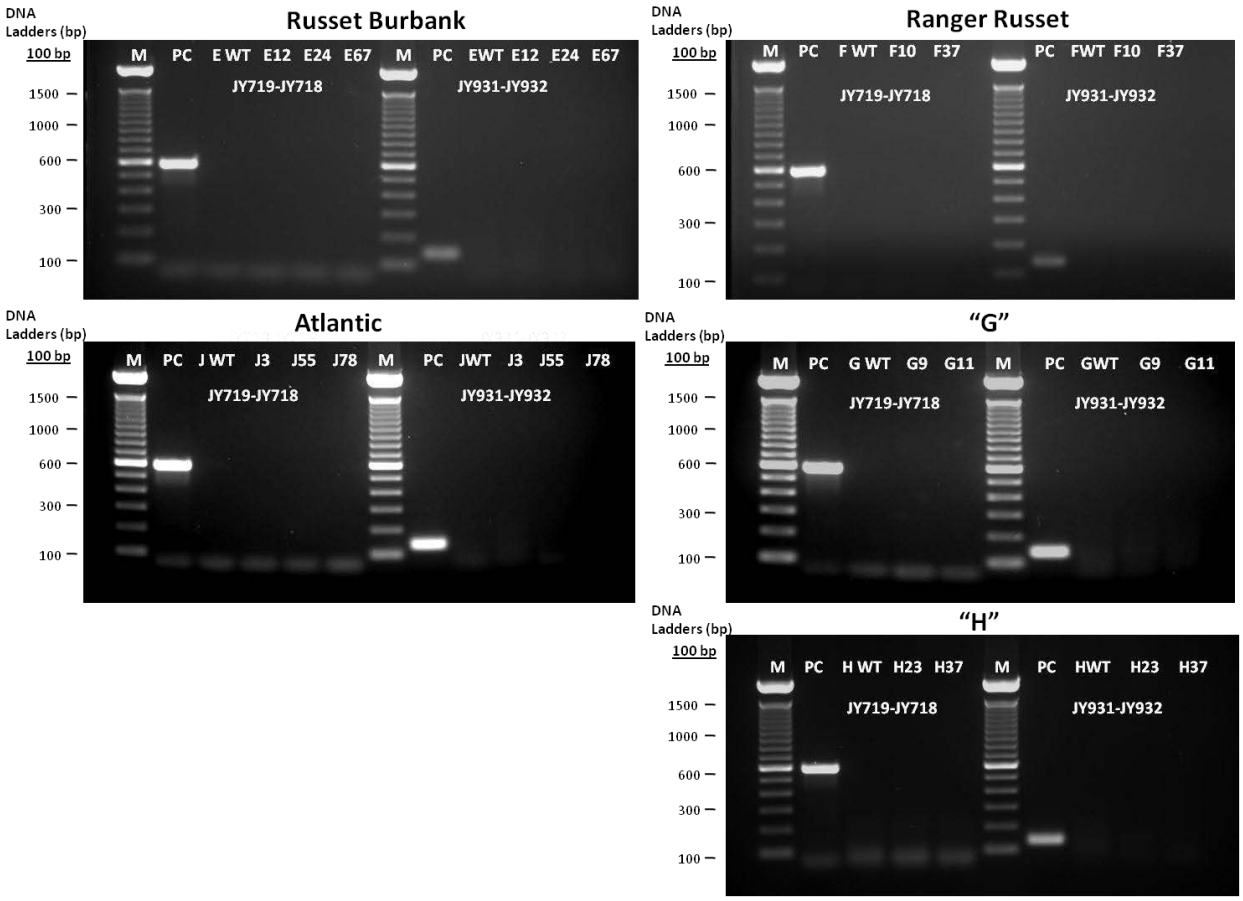
Figure 12. Confirmation of the Absence of Backbone DNA Flanking the Right Border of the DNA insert in Event H50[1]



[1]Bands on the ethidium bromide-stained gels are indicative for the presence of Right Border/backbone DNA-junctions. M = 100-bp DNA marker lane (invitrogen), PC = positive plant control, H74 not included in this submission, NC = negative control (untransformed recipient variety), primers printed on each gel.

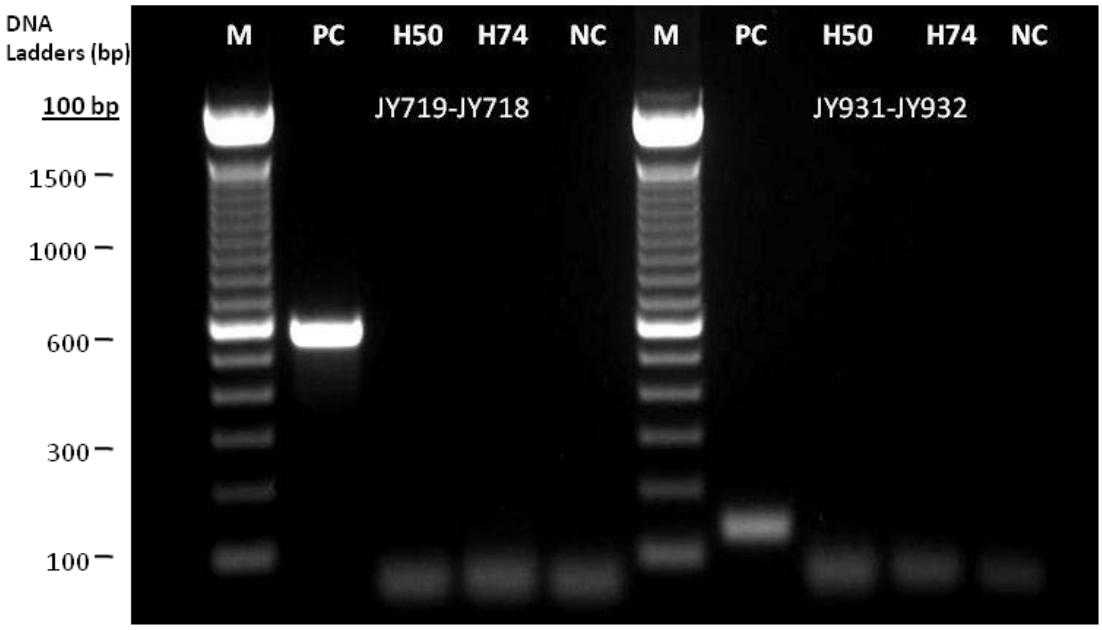
Similarly, results for amplifications with Left Border-associated primers, shown in **Figures 13** and **14**, indicate the absence of backbone DNA beyond the Left Border.

Figure 13. Confirmation of the Absence of Backbone DNA Flanking the Left Border of the DNA insert[1]



[1]Bands on the ethidium bromide-stained gels are indicative for the presence of Left Border/backbone DNA-junctions. Data for all events, except for H50, are shown (see **Figure 14** for H50). M = 100-bp DNA marker lane (invitrogen), PC = positive plant control, WT = untransformed recipient variety ("wild type", negative control), primers printed on each gel. E67, G9, and H23 not included in the submission.

Figure 14. Confirmation of the Absence of Backbone DNA Flanking the Left Border of the DNA insert in Event H50[1]



[1]Bands on the ethidium bromide-stained gels are indicative for the presence of Left Border/backbone DNA-junctions. M = 100-bp DNA marker lane (invitrogen), PC = positive plant control, H74 = not included in this submission, NC = negative control (untransformed plant), primers printed on each gel.

MATERIALS AND METHODS

Plant material. Plants used for DNA gel blot analysis were grown for two months in Sunshine mix-1 (www.sungro.com) in two-gallon pots in a greenhouse that was controlled for temperature (18°C minimum/27°C maximum) and light (16-h photoperiod with an intensity of ~1500 µmol/m2/s).

Use of the isopentenyl isomerase (*ipt*) gene as vector backbone marker. Only shoots that were phenotypically-indistinguishable from controls (not overproducing cytokinins) were selected during the transformation process and allowed to develop into whole plants.

DNA Isolation. For plant DNA isolation, 1.0 g of young potato leaves was ground into a fine powder in a mortar using 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 until ready to process. 10 ml extraction buffer (0.35 M Sorbitol, 0.1 M Tris-HCl, pH8.0, 0.05M EDTA) was added to, and mixed with, the powder, and the resulting suspension was centrifuged at 3,000 rpm for 15 min at room temperature. The pellet was thoroughly re-suspended in 2 ml extraction buffer containing 200 µg RNase A. After incubating the resuspended DNA at 65^{0}C for 20 min with 2 ml of nuclear lysis buffer (0.2 M Tris-HCl pH7.5, 0.005 M EDTA pH 8.0 and 20 mg/ml CTAB Hexadecyl Trimetyl Ammonium Bromide) and 800 µl of 5% Sarcosyl, it was mixed with an equal volume of chloroform : isoamyl alcohol (24:1), vortexed for about 1 min, and centrifuged at 3000 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 a spectrophotometer at an OD of 260 nm, whereas 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. In some cases, an alternative method was used to isolate DNA. The ground tissue from 0.7 g young leaves was mixed with 7 ml CTAB buffer (2% CTAB, 1.4 M NaCl, 0.1 M pH 8.0 Tris-HCl, 20 mM pH8.0 EDTA, 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 3000 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 dried in air and dissolved in TE buffer. The quality and concentration of DNA was determined spectrophotometrically and by running a sample on a 0.8% agarose gel. Control and event DNA was always extracted with the same method for a specific comparison.

DNA Gel Blot Preparation. Digested DNA was loaded on a large 0.7% agarose gel (170 ml) containing 0.5X Tris-borate-EDTA (TBE) buffer and 3-5 µl ethidium bromide (10 mg/ml) and run at 25 volts for 18 hrs. The gel was photographed using a gel documentation system from Alpha Innotech (Santa Clara, California), and then depurinated by submerging it into 0.25 N HCl for 20 min. After subsequent denaturing in 0.5 M NaOH /1.5 M NaCl for 2X15 min and neutralization in 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 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.

Probe Preparation. Nylon filters carrying *Eco*RI-digested DNA of the events were hybridized with six different probes, each of which represented an approximately 1-kb DNA fragment of the vector backbone (see **Table 2** for primer sequences and **Figure 3** for the linear arrangements of the probes described in **Table 2**). Untransformed potato varieties were used as negative controls, and TT4, T130 and G23 events carrying most or all of the pSIM1278 vector provided positive controls.

Table 2. Backbone Probes

Backbone Probe Nr.	Size	pSIM1278 Coordinates	Forward Primer	Reverse Primer
1	1,473-bp	9,957-11,429	ACTAGTTGTGAATAAGTCGCTGTG	ATCGGAATCGACTAACAGAACAT
2	1,514-bp	11,398-12,911	CCGGGGCCGATGTTCTGTTAG	GCTCGCCGGCAGAACTTGAG
3	1,588-bp	12,860-14,447	GCCGCGTGTTCCGTCCACAC	CCTGTCGGGTTTCGCCACCT
4	1,660-bp	14,420-16,079	CAAGTCAGAGGTGGCGAAAC	CTTTATGCTCATTGGGTTGAGTA
5	1,074-bp	16,396-17,469	AGTCCACCCGAAATATAAACAAC	GGTATGGACCTGCATCTAATTTTC
6	834-bp	18,633-19,466	GCTCTAATATAGCGCATTTCAAG	GCTTCCAGCCAGCCAACAGCTC

The labeling of 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 10 x Hotmaster Taq Buffer, 2-5 µl 10 uM forward primer, 2-5 µl 10 µM reverse primer, 5 µl DIG labeled dNTP (Roche), 10 ng plasmid template, 0.75 µl Hotmaster Taq polymerase, and water. 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 (it always ran slower than control PCR product). The probe was denatured before use by incubating the probe at 100°C for 5 min, placing on ice for 2 min.

Southern Blot 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 hrs in a bottle in a standard hybridization oven (Amerex Instruments Inc.) at 20-25 rpm. Hybridization was carried out by replacing the prehybridization buffer with a fresh amount of the same preheated solution, now containing 25-50 µl denatured DIG labeled probe, and continuing the incubation at 42°C, 20-25 rpm for about 16 hrs. The hybridization solution could be store at -20°C and reused up to 3 times. The reused hybridization solution was heated at 68°C for 10 minutes before use to remove archives from previous runs.

Southern Blot 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^{0}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 brief 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 hrs 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 a plastic film and exposed to the Z-ray film in the dark. Depending on the experiment multiple exposures were taken from 30 sec to 30 min. The films were developed with Konica SRX-101A Z-ray film developer. The developed films were scanned to obtain the final images.

PCR-Based Identification of Vector Backbone sequences. The standard 30 µl PCR reaction mixture consisted of 3 µl of 10X PCR buffer, 0.6 µl of 10 mM dNTP, 0.6 µl of 10 µM forward primer, 0.6 µl of 10 µM reverse primer, 100 ng of genomic DNA template, with 0.2 µl of HotMaster Taq polymerase (Fisher BioReagents). The PCR was carried out under the following amplification conditions: 1 cycle of 3 min at

95 °C followed by 40 cycles of 30 sec at 94 °C, 30 sec at 55°C, 45 sec at 68 °C, and finishing with 10 min at 68 °C.

Primers were designed to amplify fragments indicative of (1) junctions between the DNA insert border regions and flanking backbone DNA or (2) regions within the backbone DNA that flank the DNA insert. The primer pair JY725-JY726 amplified a 377-bp fragment comprising the junction at the Right Border region and flanking backbone, and primers JY915-JY749 were used to amplify a 215-bp backbone fragment flanking the Right Border (see **Table 3** and **Figures 11-12**). The primer pair JY718-JY719 amplified a 587-bp fragment comprising the junction at the Left Border region and flanking backbone, and primers JY931-JY932 were used to amplify a 134-bp backbone fragment flanking the Left Border (see **Table 3** and **Figures 13- 14**). H50 was run separately on the gels to verify similarities to H74. However, H74 is not a part of this submission.

Table 3. Primers Used for Detecting Backbone
Primers Used for Detecting Backbone Adjacent to Left Border of pSIM1278

Name	Sequence	Position in pSIM1278	product	Backbone bp
JY719	GAGCTGTTGGCTGGCTGGAAG	19445-19465, in backbone		
JY718	GTTGGAAATCAATTATCACTGAG	349-371, in AGP promoter	587 bp	24
JY931	CGAGATCATCCGTGTTTCAA	19278-19297, in backbone		
JY932	GATACAGGCAGCCCATCAGT	19392-19411, in backbone	134 bp	134

Primers Used for Detecting Backbone Adjacent to Right Border of pSIM1278

Name	Sequence	Position in pSIM1278	product	Backbone bp
JY725	GCTTCCCGTATACAACATAACATG	9621-9644, in GBSS promoter		
JY726	GATCTCAAACAAACATACACAGCG	9974-9997, in backbone	377 bp	41
JY915	ACTAGTTGTGAATAAGTCGCTGTG	9957-9980, in backbone		
JY749	GAAGCCGACTGCACTATAGCAG	10150-10171, in backbone	215 bp	215

Figure 15. PCR Probe Positions for Backbone Detection

CONCLUSIONS

Event Ranger Russet F10 lacks any detectable vector backbone DNA.
Event Ranger Russet F37 lacks any detectable vector backbone DNA.
Event Russet Burbank E12 lacks any detectable vector backbone DNA.
Event Russet Burbank F10 lacks any detectable vector backbone DNA
Event Atlantic J3 lacks any detectable vector backbone DNA.
Event Atlantic J55 lacks any detectable vector backbone DNA.
Event Atlantic J78 lacks any detectable vector backbone DNA.
Event G11 lacks any detectable vector backbone DNA.
Event H37 lacks any detectable vector backbone DNA.
Event H50 lacks any detectable vector backbone DNA.

A robust triple confirmation process was utilized to prove the lack of vector backbone in any of the 10 events, which are the focus of this deregulation petition. These studies confirm that the events presented for deregulation do not contain backbone DNA. They indicate that further review of the safety of the vector DNA is not needed as this DNA is not present in the Innate™ events.

REFERENCES

Cambia (2012) pCambia Vectors. http://www.cambia.org/daisy/cambia/585.html (Accessed 7 May 2012).

Courvalin P, Weisblum B, Davies J (1977) Aminoglycoside-modifying enzyme of an antibiotic-producing bacterium acts as a determinant of antibiotic resistance in Escherichia coli. P Natl Acad Sci USA, 74: 999-1003.

Garbarino JE, Belknap WR (1994) Isolation of a ubiquitin-ribosomal protein gene (ubi3) from potato and expression of its promoter in transgenic plants. Plant Mol Biol, 24: 119-127.

Richael CM, Kalyaeva M, Chretien RC, Yan H, Adimulam S, Stivison A, Weeks JT, Rommens CM (2008) Cytokinin vectors mediate marker-free and backbone-free plant transformation. Transgenic Res 17: 905-917.

Smigocki AC, Owens LD (1988) Cytokinin gene fused with a strong promoter enhances shoot organogenesis and zeatin levels in transformed plant cells. P Natl Acad Sci USA, 85: 5131-5135.

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.

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Appendix 3
Evidence for Stability of the Inserted DNA

INDEX

INTRODUCTION

The stability of the DNA inserts was evaluated in the original transformants and again in propagated plant material using both DNA gel blot hybridization and a trait evaluation assay. These studies were carried out to ensure that the desired genetic changes in events remained stable over multiple clonal cycles and expressed the traits consistently over generations. Inconsistent trait expression generationally might be triggered by rare recombination events or could also be caused by methylation. Because potatoes are clonally propagated, standard assessments for sexually propagated crops were not directly applicable. Tubers rather than seeds were used to define subsequent generations since tubers are what is commercially planted. Results of DNA blot hybridization demonstrate consistent bands were present in three generatiuons of plants, indicating stability of the traits. Further evidence for stability was obtained by confirming trait efficacy in three generations of plants (tubers) using a catechol assay for polyphenoly oxidase activity by visually evaluating the darkening of tuber flesh.

In some cases, Southern blots shown in this appendix will use the same probes and enzyme digests as found in other studies (for example, see **Appendix 1. Characterization of Inserted DNA**). The observations may differ, depending upon the conditions under which the gels were run. Therefore all bands described in the tables representing Southern blot data in this appendix refer to the gels presented within this appendix.

ABBREVIATIONS and DEFINITIONS

G0	Initial generation of tubers utilized from tissue culture
G1	First generation field grown plant (after tissue culture)
G2	Second generation field grown plant
G3	Third generation field grown plant
wt	“wild type”, this term is used to refer to the untransformed control of a given variety
MII	Molecular size marker used in lane 1 of each 0.7% agarose gel (approximate range 2.0-23.1 kb)
MVII	Molecular size marker used in lane 2 of each 0.7% agarose gel (approximate range 1.2-7.7 kb)

RESULTS

DNA Insert Stability in Ranger Russet Events F10 and F37

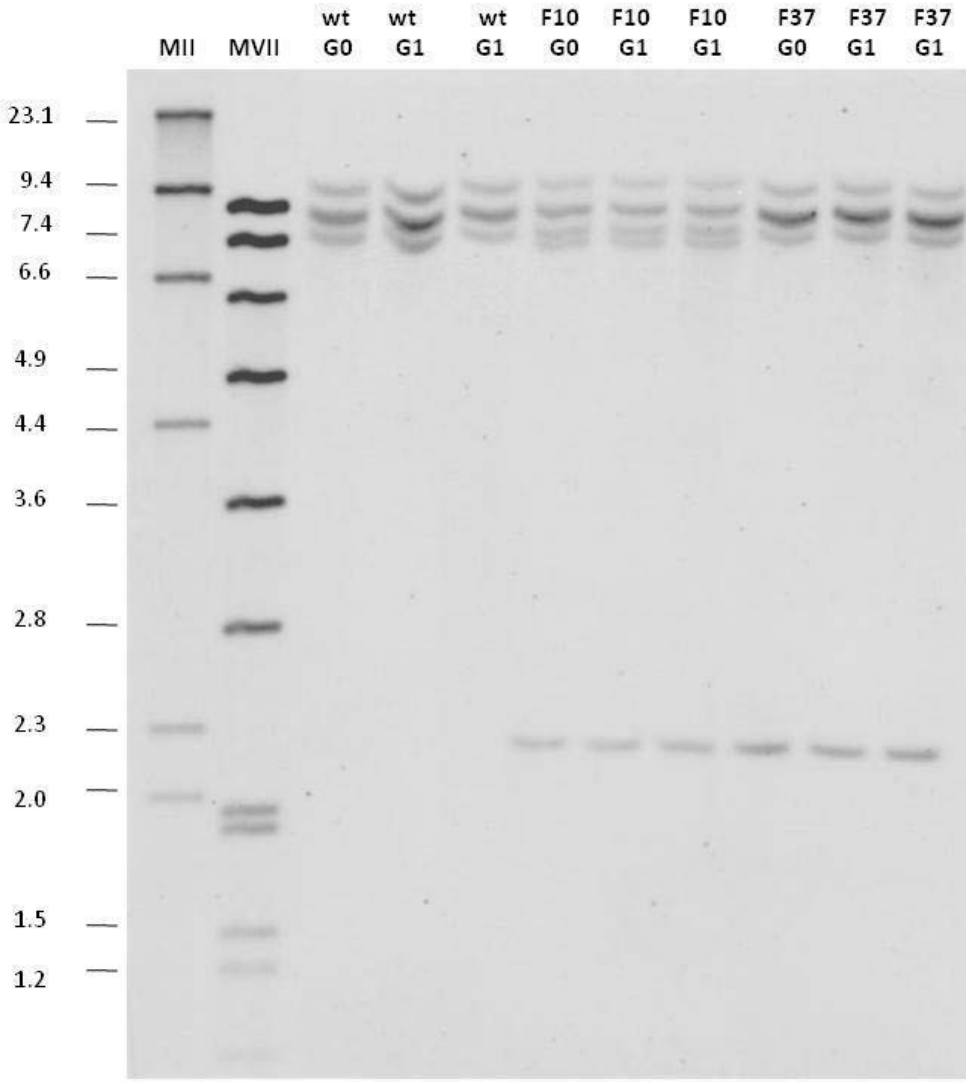
The GBS and AGP probes (see **Figure 38** in Materials and Methods) were hybridized with DNA of the Ranger Russet controls and events F10 and F37 and evaluated over 3 generations. Hybridization was used to visualize three common bands present in the unmodified control Ranger Russet variety (wt) and the F10 and F37 events. The common bands were visualized on 0.7% agarose gels with sizes of 7.4 kb, 7.7 kb, and 9.4 kb for the GBS probe and 1.2 kb, 4.9 kb, and 6.2 kb for the AGP probe (**Figures 1, 2, 3, 4, 5, and 6**).

Two additional bands hybridized with the GBS probe were observed in F10 and F37, one 2.3 kb band indicative of an internal DNA insert fragment and the other one a junction fragment (7.1 kb for F10 and 7.7 kb for F37). These were observed in all G0, G1, G2, and G3 samples, indicating that the inserts of the original transformant (G0) remained stable into the first, second, and third vegetative generations (**Figures 1, 2, and 3**). The AGP probe visualized both an IB (internal band) of 2.3 kb and a JB (junction band) of 6.5 kb for F37 in G0, G1, G2, and G3 (**Figures 4, 5, and 6**). Additionally the AGP probe hybridized with a 2.6 kb fragment for all F10 samples (**Figures 4, 5, and 6**).

Additional evidence for DNA insert stability and black spot bruise efficacy in Ranger Russet events F10 and F37 was obtained with a catechol assay for Ppo activity. The results with G2 tubers showed all Ranger Russet F10 and F37 tubers remained uncolored, indicative of resistance to black spot bruise (**Figure 7**) through *Ppo5* gene silencing.

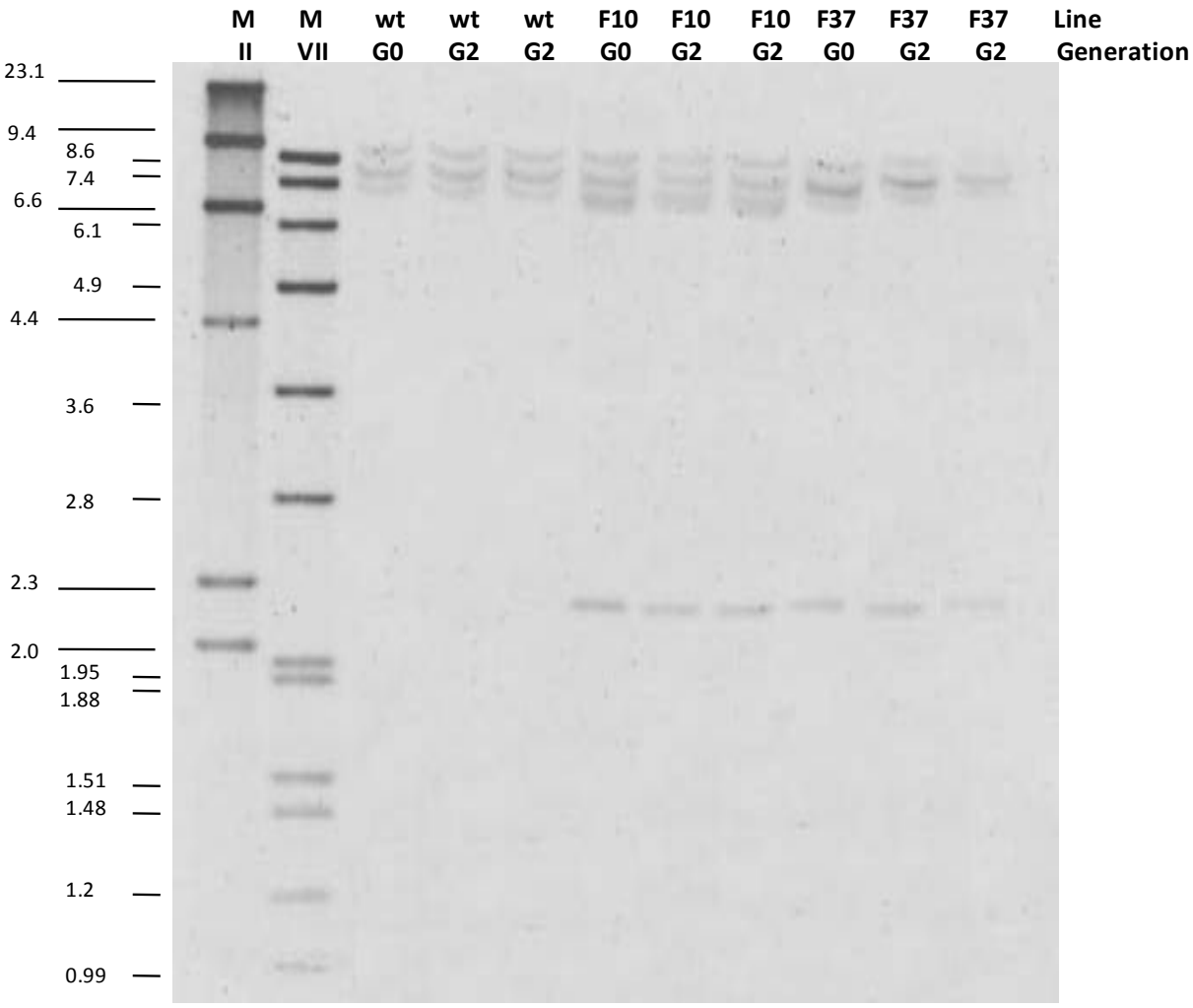
Overall, hyridization (**Figures 1, 2, 3, 4, 5, and 6**) with the GBS and AGP probes shows that events F10 and F37 had consistent bands over multiple generations by comparing G0 with G1, G2 and G3 samples. Also, the visual assay for PPO with catechol confirmed stability of *Ppo5* gene silencing (**Figure 7)** from the initial generation through G2.

Figure 1. F10 and F37: DNA Insert Stability in G0 and G1 Samples as Visualized with a GBS Probe



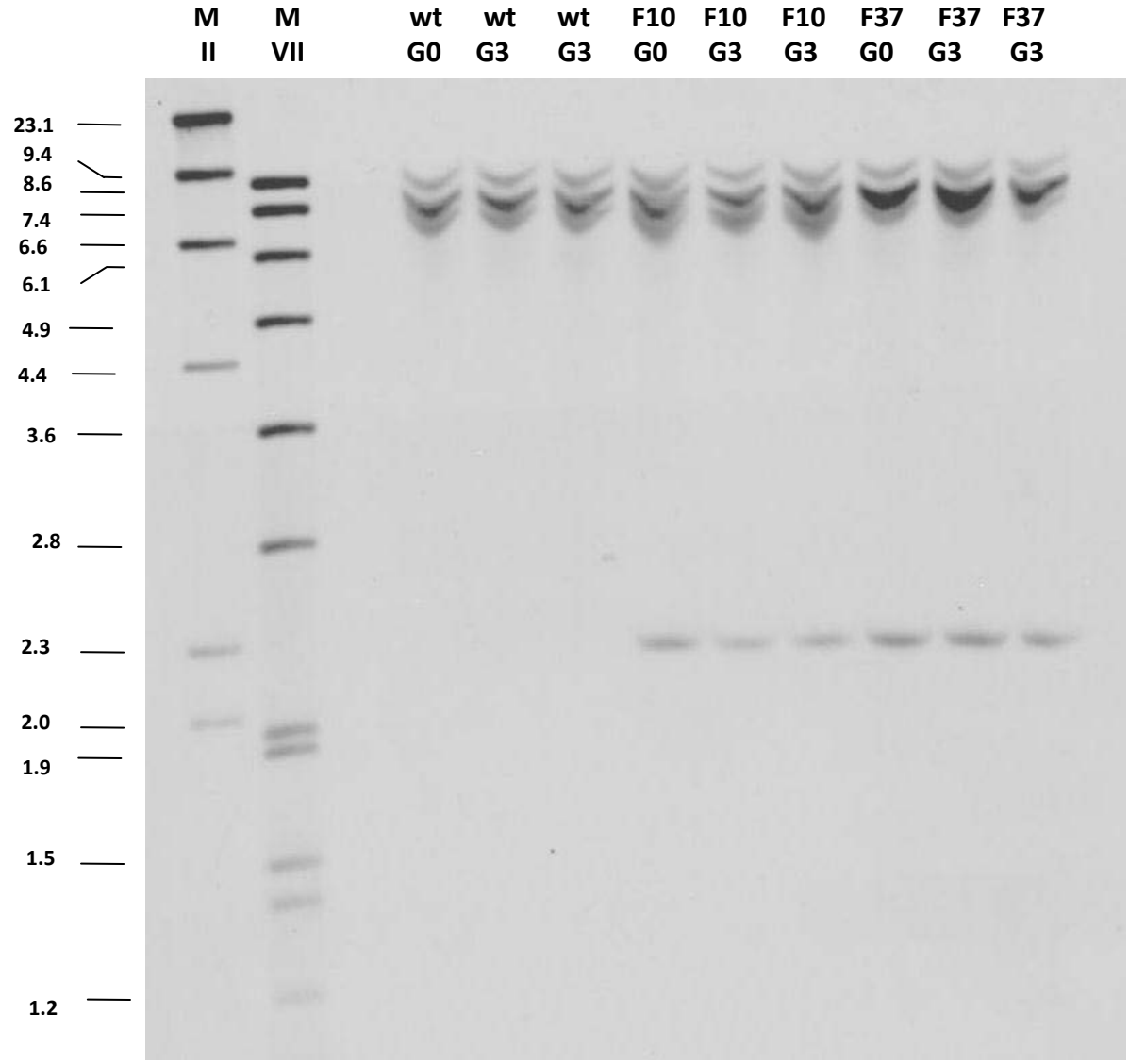
Ranger Russet untransformed controls (wt), F10, and F37 all show consistency with 7.1 kb, 7.7 kb, and 9.4 kb size bands in the G0 and G1 plants. Additionally, F10 and F37 share a band the size of 2.3 kb in both the G0 and G1 plants.

Figure 2. F10 and F37: DNA Insert Stability in G2 Samples as Visualized with a GBS Probe



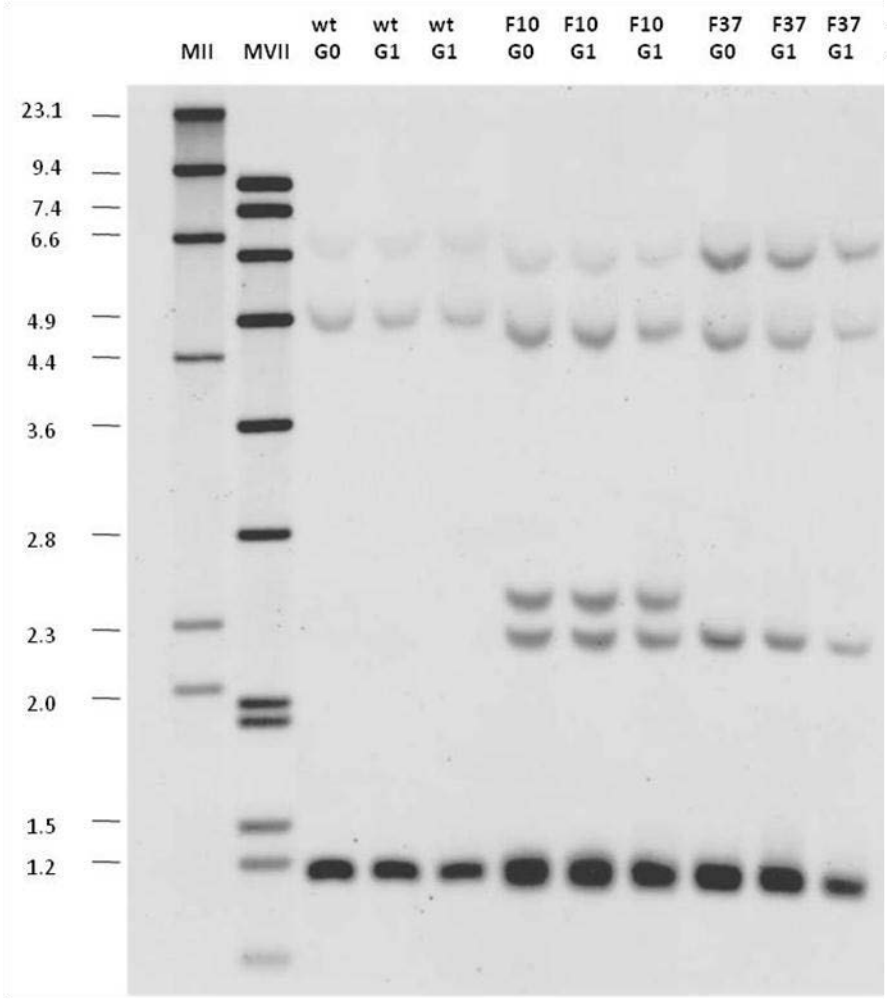
Ranger Russet untransformed controls (wt), F10, and F37 all show consistency with 7.1 kb, 7.7 kb, and 9.4 kb size bands in the G0 and G2 plants. Additionally, F10 and F37 share a band the size of 2.3 kb in both the G0 and G2 plants.

Figure 3. F10 and F37: DNA Insert Stability in G3 Samples as Visualized with a GBS Probe



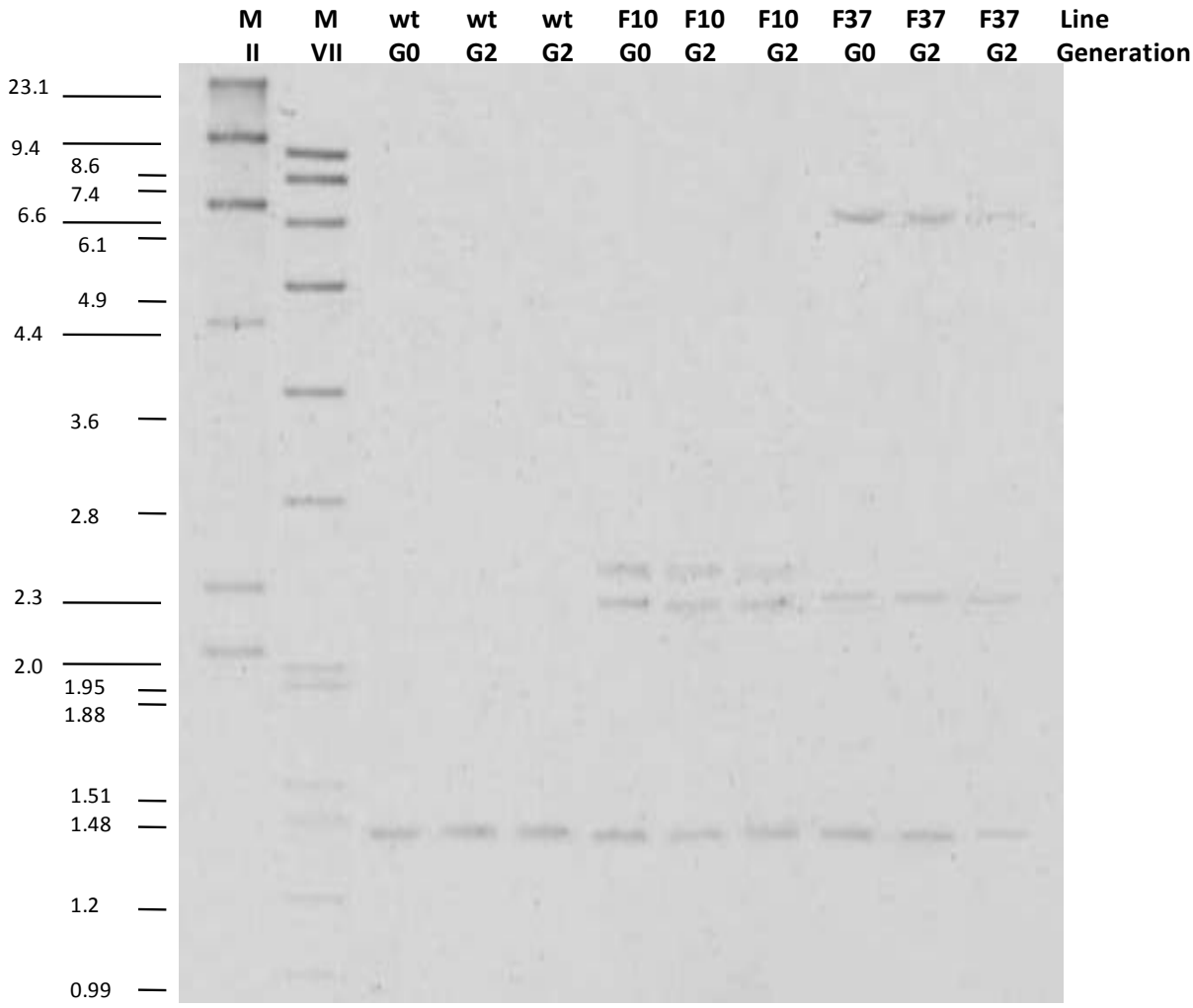
Ranger Russet untransformed controls (wt), F10, and F37 all show consistency with 7.1 kb, 7.7 kb, and 9.4 kb size bands in the G0 and G3 plants. Additionally, F10 and F37 share a band the size of 2.3 kb in both the G0 and G3 plants.

Figure 4. F10 and F37: DNA Insert Stability in G0 and G1 Samples as Visualized with an AGP Probe



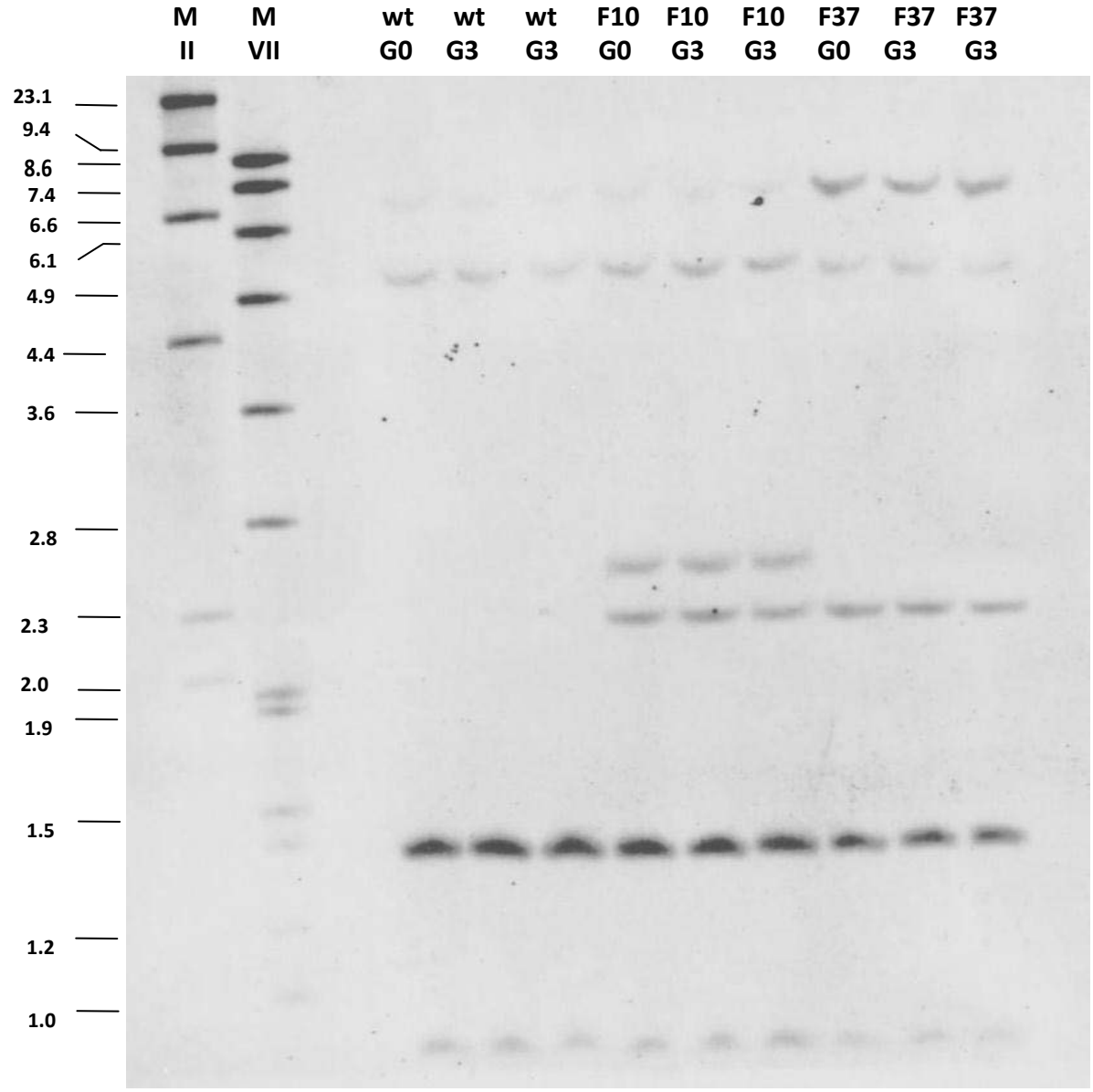
Ranger Russet untransformed controls (wt), F10, and F37 all show consistency with 1.2 kb, 4.9 kb, and 6.5 kb size bands in the G0 and G1 plants. F10 and F37 share a band the size of 2.3 kb in both the G0 and the G1 plants. F10 also has a band the size of 2.5 kb which is consistent in the G0 and G1 plants.

Figure 5. F10 and F37: DNA Insert Stability in G2 Samples as Visualized with an AGP Probe



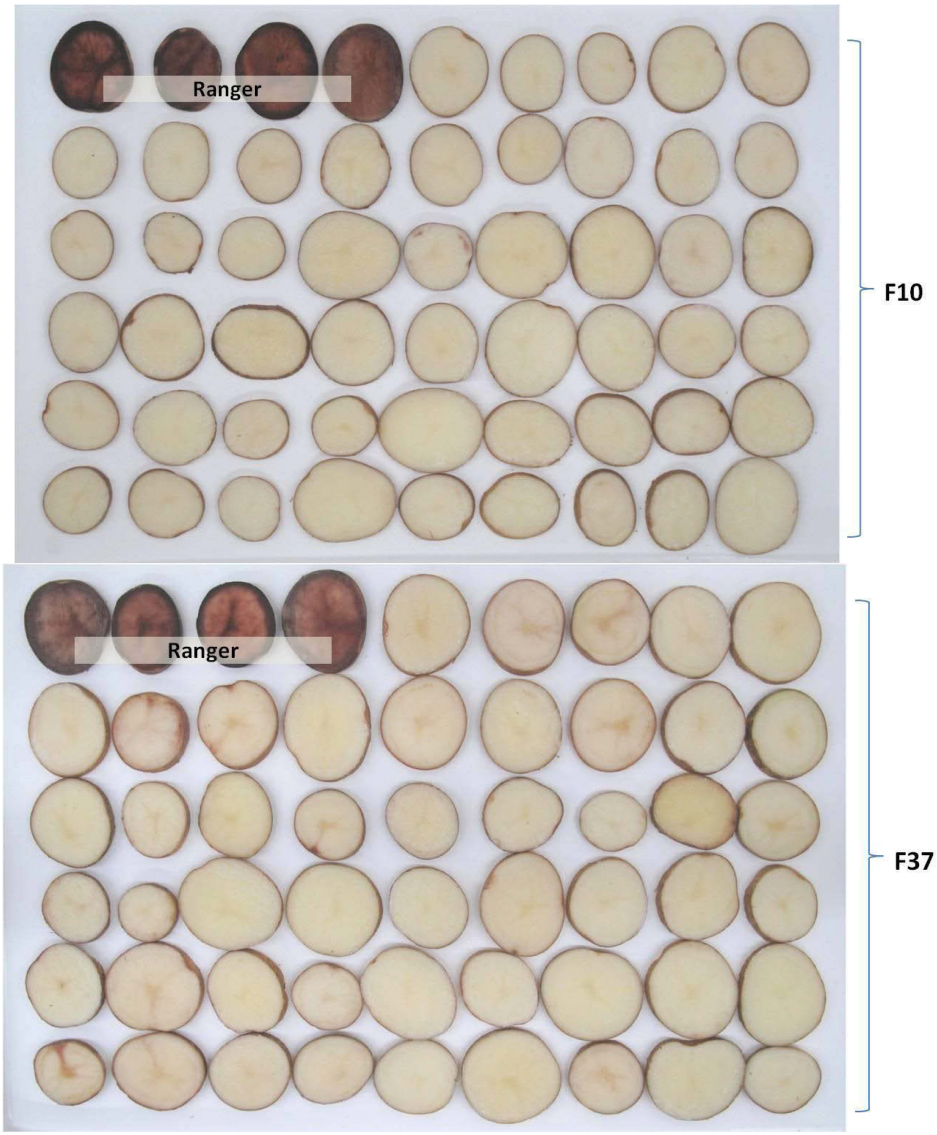
Ranger Russet untransformed controls (wt), F10, and F37 all show consistency with 1.2 kb, 4.9 kb, and 6.5 kb size bands in the G0 and G2 plants. F10 and F37 share a band the size of 2.3 kb in both the G0 and the G2 plants. F10 also has a band the size of 2.5 kb which is consistent in the G0 and G2 plants.

Figure 6. F10 and F37: DNA Insert Stability in G3 Samples as Visualized with an AGP Probe



Ranger Russet untransformed controls (wt), F10, and F37 all show consistency with 1.2 kb, 4.9 kb, and 6.5 kb size bands in the G0 and G3 plants. F10 and F37 share a band the size of 2.3 kb in both the G0 and the G3 plants. F10 also has a band the size of 2.5 kb which is consistent in the G0 and G3 plants.

Figure 7. Assay for Sustained *Ppo5* Gene Silencing in G2 Tubers of F10 and F37



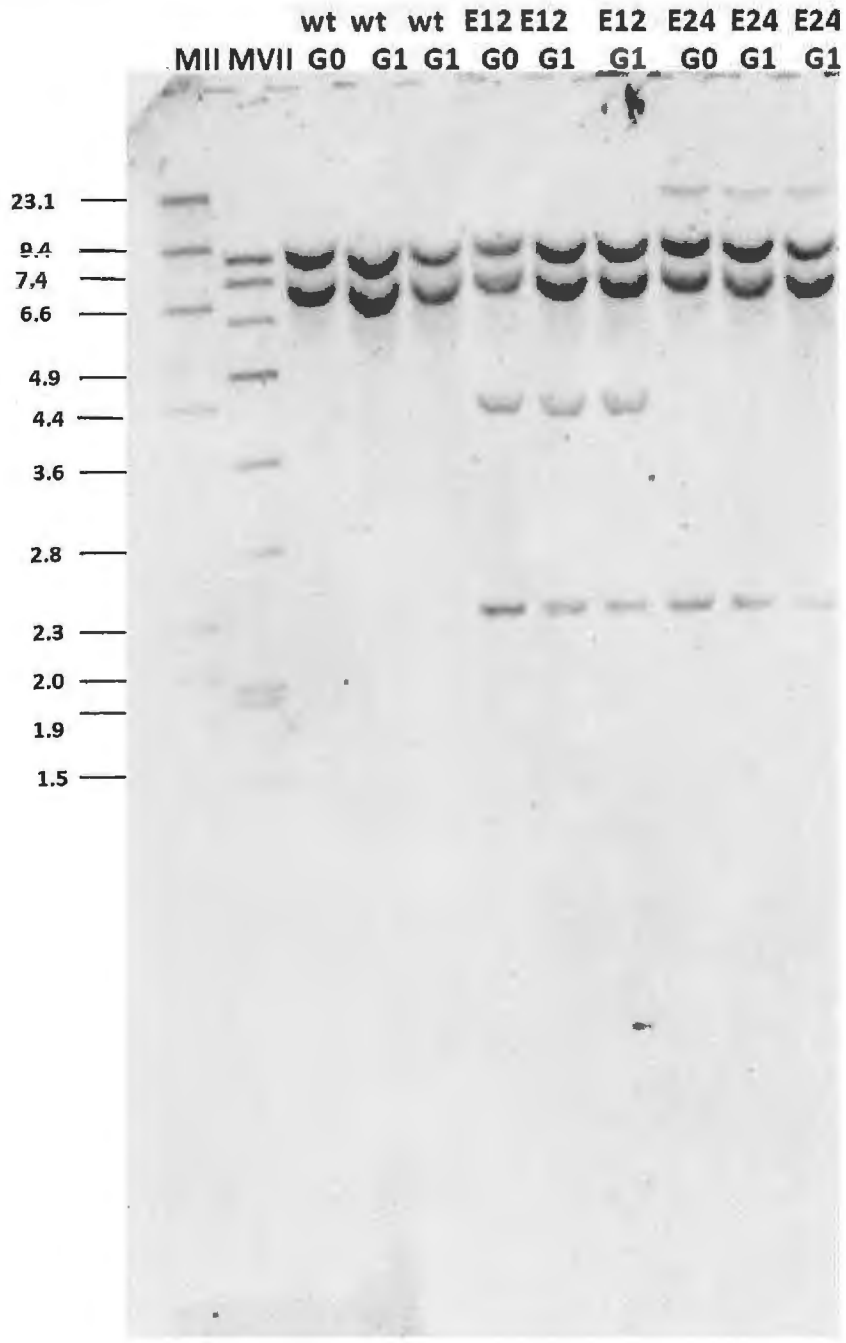
DNA Stability in Russet Burbank Events E12 and E24

Hybridization of DNA of Russet Burbank E12 and E24 plants visualizes two common bands with the GBS probe (7.5 and 8.5 kb) (**Figures 8, 9, and 10**) and three with the AGP probe (1.5, 4.4 and 5.2 kb)in all lanes (**Figures 11, 12, and 13**). These bands are indicative of the unmodified genome. Two additional bands in DNA of all event material, one 2.3 kb band indicative of an internal DNA insert fragment and the other one representing a DNA insert junction fragment (4.5 kb for E12 with GBS, ~23 kb for E24 with GBS, 2.2 kb for E12 with AGP, and ~20 kb for E24 with AGP), indicate that the inserts of the original transformant (G0) remained stable into the first, second, and third vegetative generations G1, and G2, and G3 (**Figures 8, 9, and 10**).

Additional evidence for DNA insert stability in events E12 and E24 was obtained with the catechol assay for Ppo activity, using tubers from the second generation. As shown in **Figure 14**, the G2 tubers did not brown, except for the untransformed control material. This demonstrated that the events for each variety had retained their ability to silence the *Ppo5* gene in tubers, after two years of field trials. Preparation of the field trial material had taken the events through repeated tissue culture and vegetative generations without affecting the stability of the inserted genetic material. Note: Slight differences in flesh color of the event tubers is from natural variation and is not related to Ppo silencing.

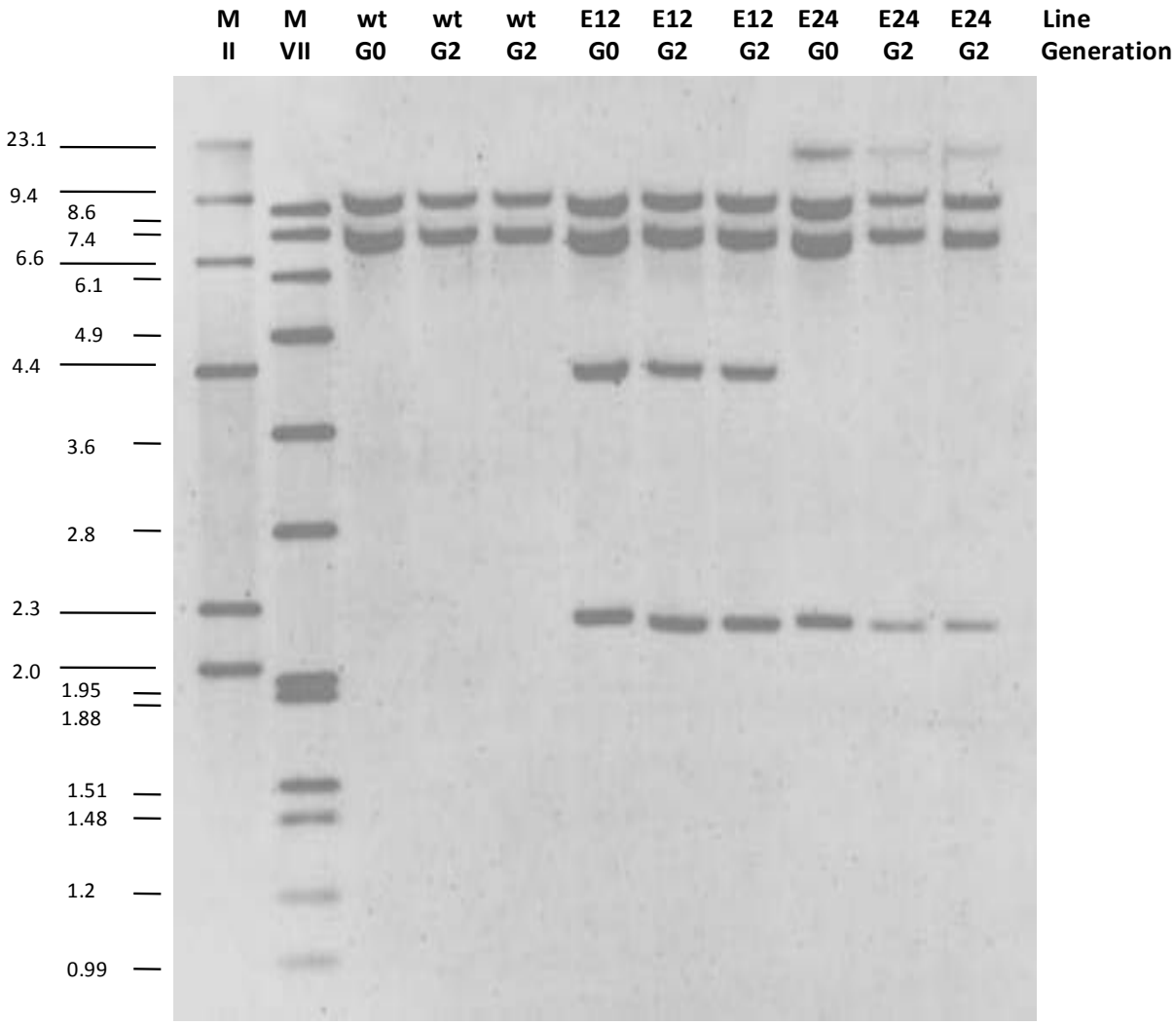
Overall, hyridization (**Figures 8, 9, 10, 11, 12, and 13**) with the GBS and AGP probes shows that events E12 and E24 had consistent bands over multiple generations by comparing tubers of G0 with G1, G2 and G3. Also, the visual assay for PPO with catechol (**Figure 14**) confirmed stability of *Ppo5* gene silencing from the initial generation through G2.

Figure 8. E12 and E24: DNA Insert Stability in G0 and G1 Samples as Visualized with a GBS Probe



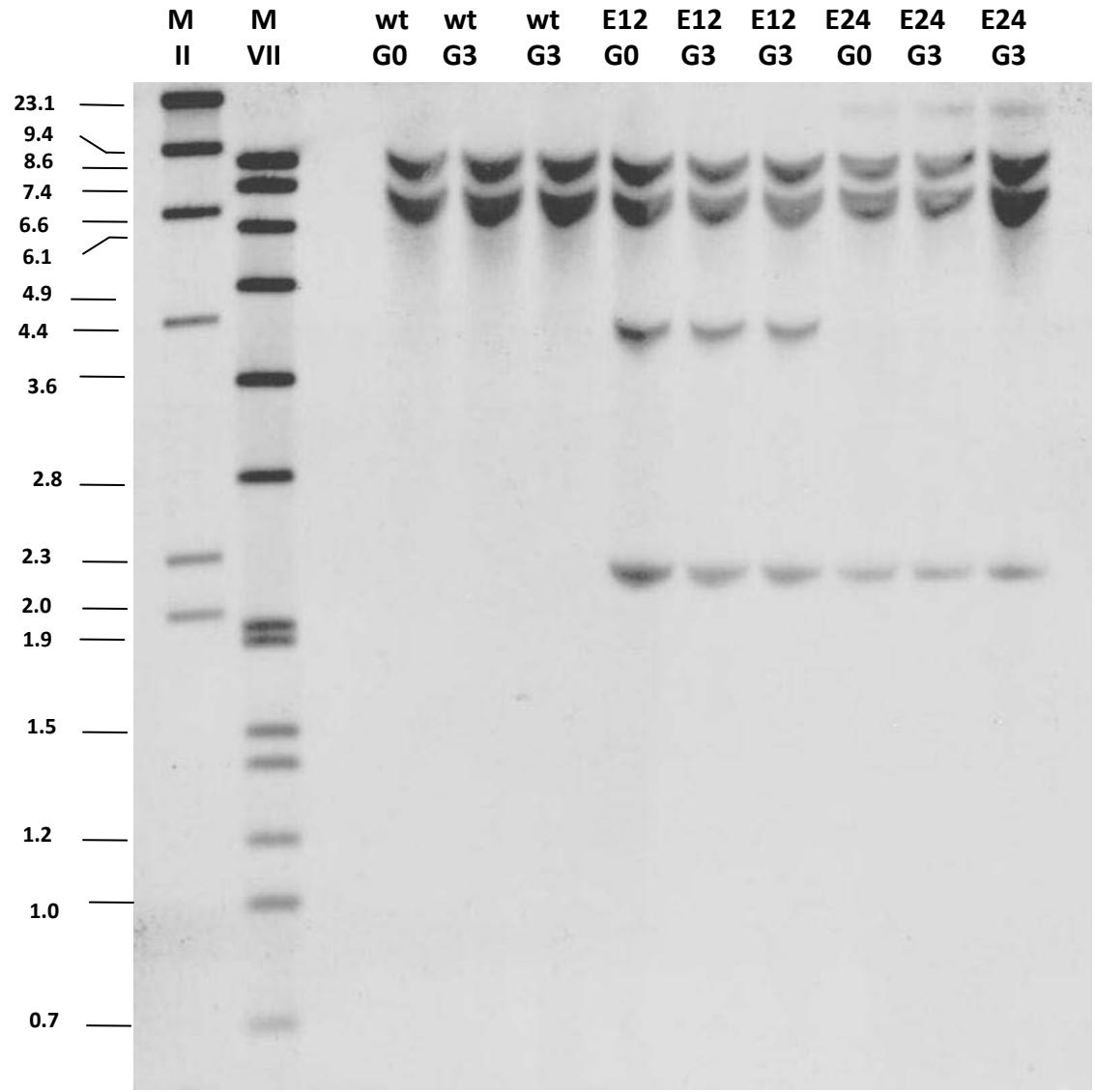
Russet Burbank untransformed controls (wt), E12, and E24 all show consistency with 7.5 kb and 8.5 kb size bands in the G0 and G1 plants. E12 and E24 share a band the size of 2.3 kb in both the G0 and the G1 plants. E12 has a 4.5 kb band and E24 has a ~23 kb band in the G0 and G1 plants.

Figure 9. E12 and E24: DNA Insert Stability in G2 Samples as Visualized with a GBS Probe



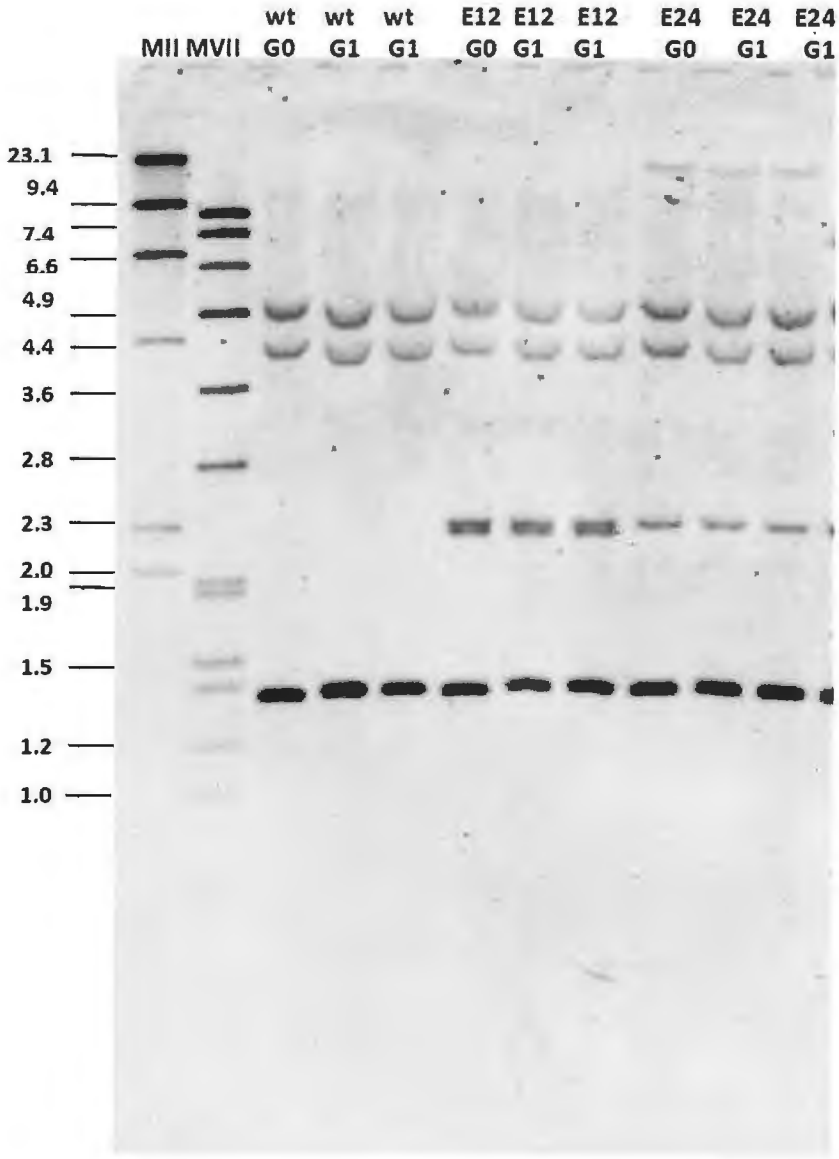
Russet Burbank untransformed controls (wt), E12, and E24 all show consistency with 7.5 kb and 8.5 kb size bands in the G0 and G2 plants. E12 and E24 share a band the size of 2.3 kb in both the G0 and the G2 plants. E12 has a 4.5 kb band and E24 has a ~23 kb band in the G0 and G2 plants.

Figure 10. E12 and E24: DNA Insert Stability in G3 Samples as Visualized with a GBS Probe



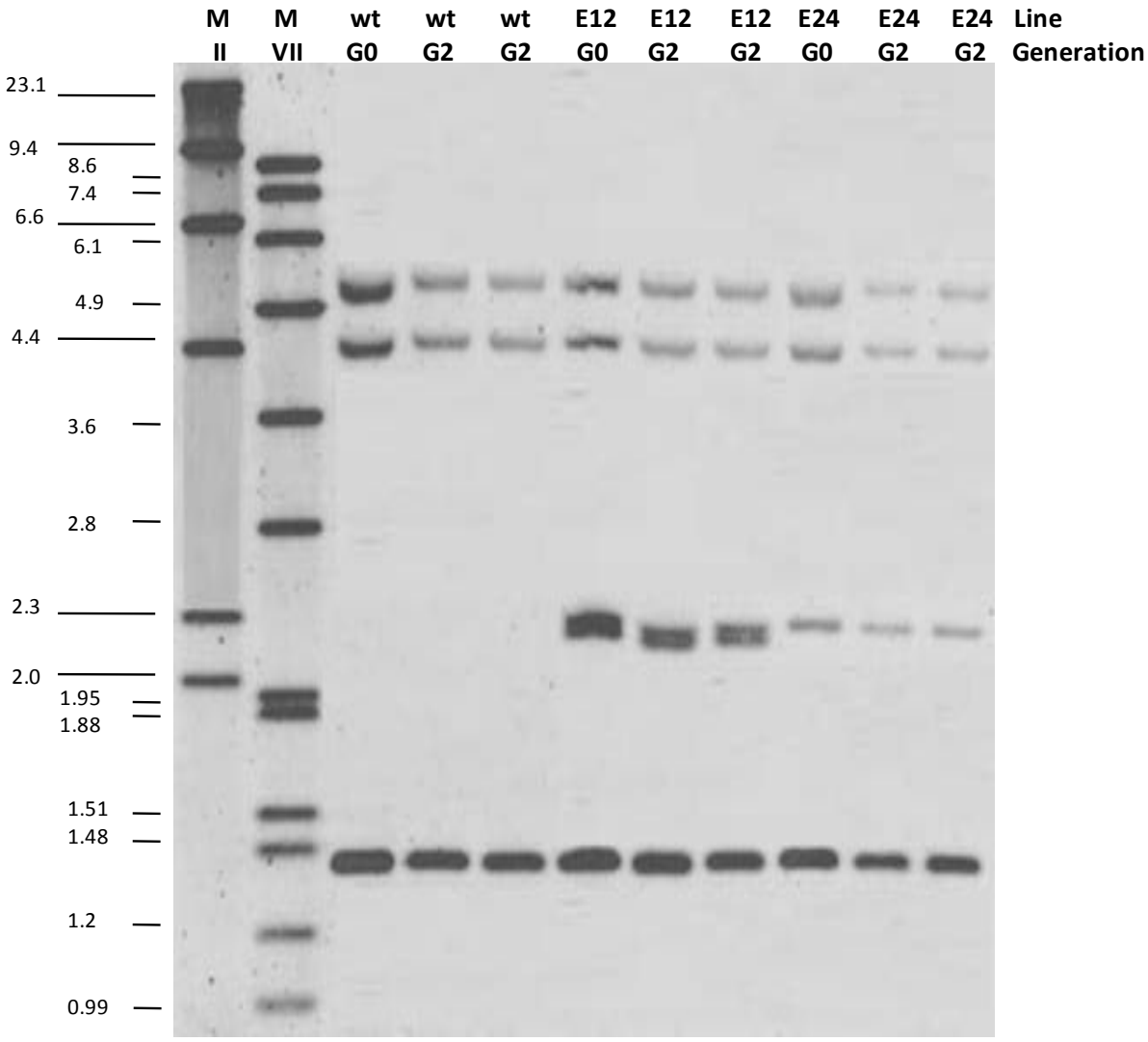
Russet Burbank untransformed controls (wt), E12, and E24 all show consistency with 7.5 kb and 8.5 kb size bands in the G0 and G3 plants. E12 and E24 share a band the size of 2.3 kb in both the G0 and the G3 plants. E12 has a 4.5 kb band and E24 has a ~23 kb band in the G0 and G3 plants.

Figure 11. E12 and 24: DNA Insert Stability in G0 and G1 Samples as Visualized with an AGP Probe



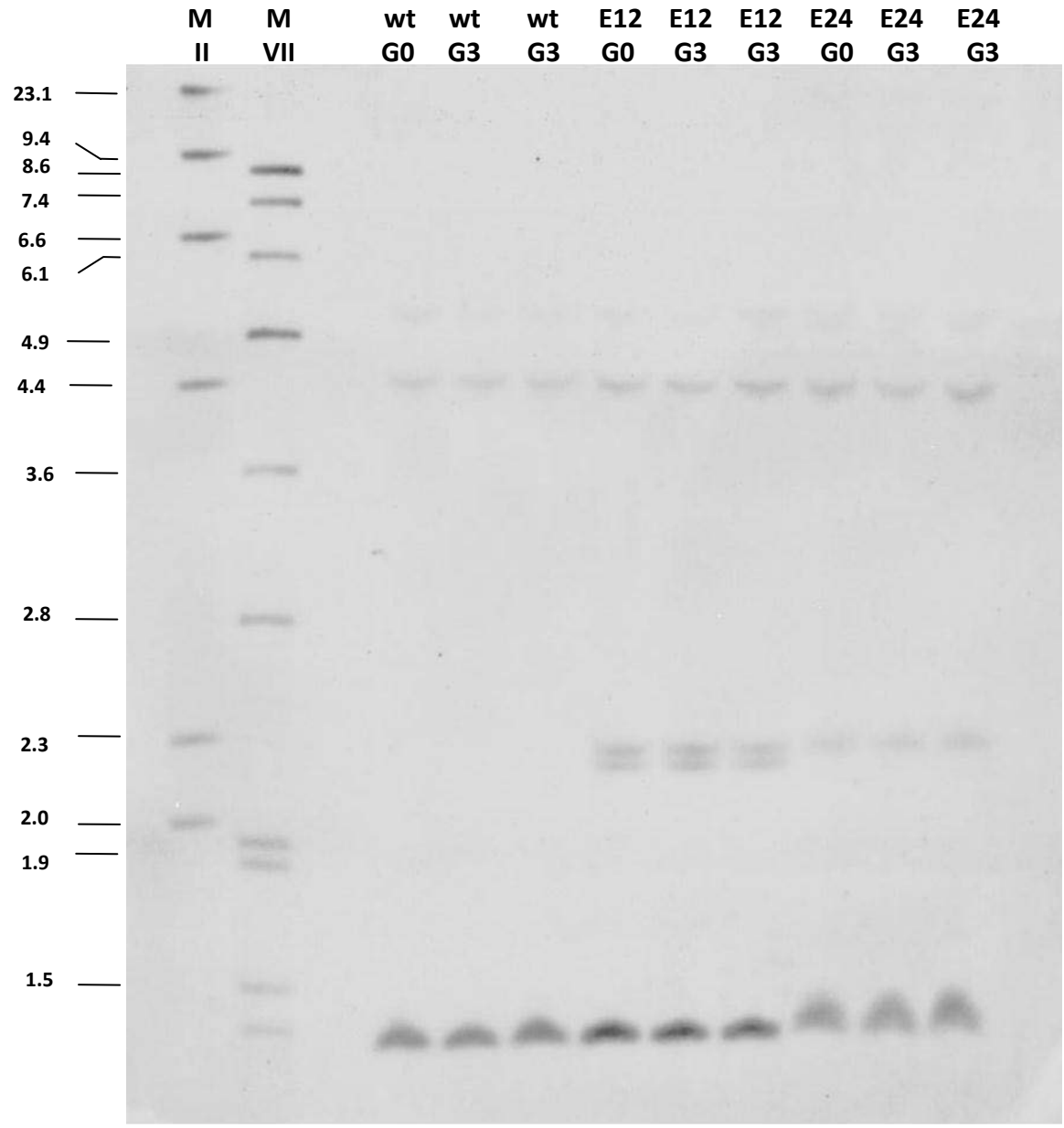
Russet Burbank untransformed controls (wt), E12, and E24 all show consistency with 1.5, 4.4, and 5.2 kb kb size bands in the G0 and G1 plants. E12 and E24 share a band the size of 2.3 kb in both the G0 and the G1 plants. E12 has a 2.15 kb band (slightly below the 2.2 kb band) and E24 has a ~20 kb band in the G0 and G1 plants.

Figure 12. E12 and E24: DNA Insert Stability in G2 Samples as Visualized with an AGP Probe



Russet Burbank untransformed controls (wt), E12, and E24 all show consistency with 1.5, 4.4, and 5.2 kb kb size bands in the G0 and G2 plants. E12 and E24 share a band the size of 2.3 kb in both the G0 and the G2 plants. E12 has a 2.2 kb band (slightly below the 2.2 kb band) and E24 has a ~20 kb band in the G0 and G2 plants.

Figure 13. E12 and E24: DNA Insert Stability in Samples as Visualized with an AGP Probe



Russet Burbank untransformed controls (wt), E12, and E24 all show consistency with 1.5, 4.4, and 5.2 kb (very light bands) size bands in the G0 and G3 plants. E12 and E24 share a band the size of 2.3 kb in both the G0 and the G3 plants. E12 has a 2.2 kb band (slightly below the 2.2 kb band) and E24 has a ~20 kb band in the G0 and G3 plants.

Figure 14. Assay for Sustained *Ppo5* Gene Silencing in G2 Tubers of E12 and E24

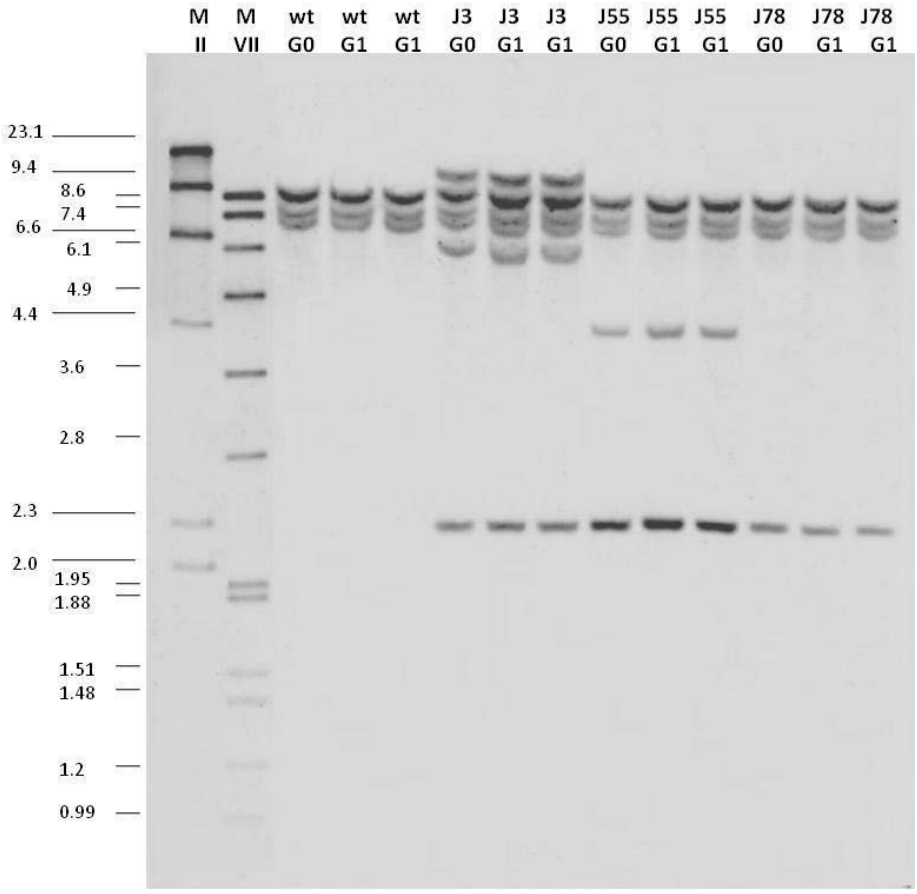
DNA Insert Stability in Events J3, J55 and J78

Hybridization of DNA of Atlantic, J3, J55 and J78 plants visualizes three common bands with the GBS probe (7.0, 7.8, and 8.6 kb) and four with the AGP probe (1.4, 2.0, 5.0, and 7.2 kb) in all lanes (**Figures 15, 16, 17, 18, 19, and 20).** These bands are indicative of DNA fragments of the unmodified genome. Three additional bands in DNA of J3, one 2.3 kb band indicative of an internal DNA insert fragment and two others representing DNA insert junction fragments (5.8 and ~12 kb with GBS and 1.6 and 5.7 kb with AGP), indicate that the inserts of the original transformant (G0) remained stable into the first, second, and third vegetative generations (**Figures 15, 16, 17, 18, 19, and 20**). Similarly, J55 contains the internal 2.3 kb internal band and additional bands (2.3 and 4.1 kb with GBS and 1.95, 2.3 and 4.8 kb with AGP) that are identical in G0, G1, G2, and G3 samples (**Figures 15, 16, 17, 18, 19, and 20**). Event J78 also contains the internal 2.3 kb DNA insert fragment in all generations and a junction fragment that only hybridizes with the AGP probe (~9 kb) (**Figures 18, 19, and 20**). There is no junction fragment with the GBS probe because the J78 insert had a DNA insert deletion of the Gbss promoter at the Left Border site (as described in the Characterization of Inserted DNA study).

The Atlantic intrangenic G2-tubers could not be assayed for Ppo activity because cut tuber surfaces of this untransformed variety do not develop a brown precipitant when exposed to catechol. It is possible that they do not produce the enzyme that catalyzes the oxidation of polyphenols. Instead, additional evidence for DNA insert stability in events J3, J55 and J78 was obtained with a PCR test using primers HD121F and HD121R (see **Figure 39** in **Materials and Methods**), which demonstrated that all Samples analyzed contained the same amplified 0.8 kb product (part of the *Asn1/ Ppo5* gene) and showing stability of the DNA insert in G2 Samples (**Figures 21 and 22**). Although G0 Samples were not available for this test, the presence of part of the *Asn1/ Ppo5* gene indicates insertion stability in G2 Samples.

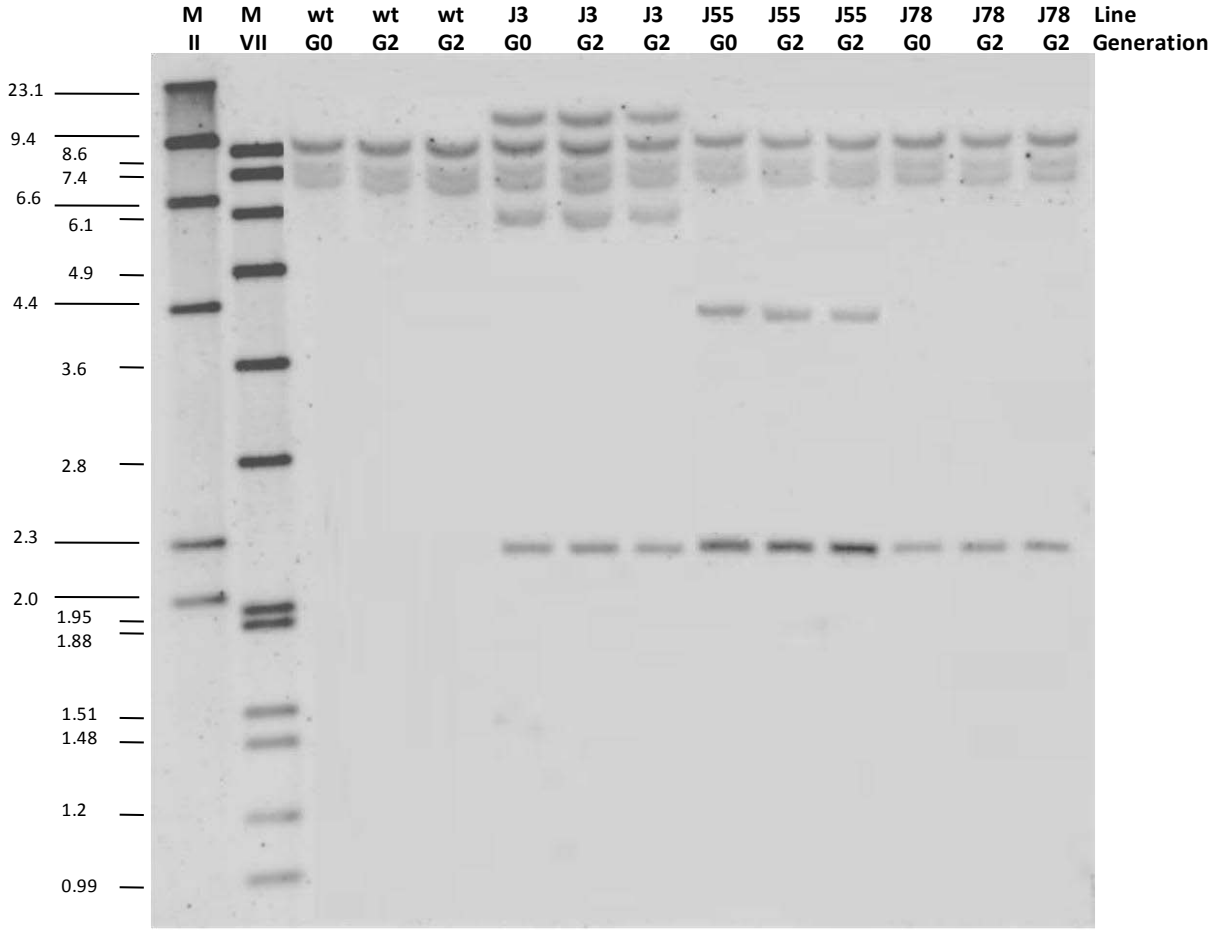
Overall, hybridization (**Figures 15, 16, 17, 18, 19, and 20**) with the GBS and AGP probes shows that events J3, J55, and J78 had consistent bands over multiple generations by comparing samples of G0 with G1, G2 and G3. Also, PCR testing (**Figures 21 and 22**) showed amplification of the same 788 bp DNA insert fragment in all G2 events, that was not found in the control Atlantic samples, indicating that the DNA insert was stable through at least the second generation.

Figure 15. Atlantic J3, J55, J78: DNA Insert Stability in G0 and G1 Samples as Visualized with a GBS Probe



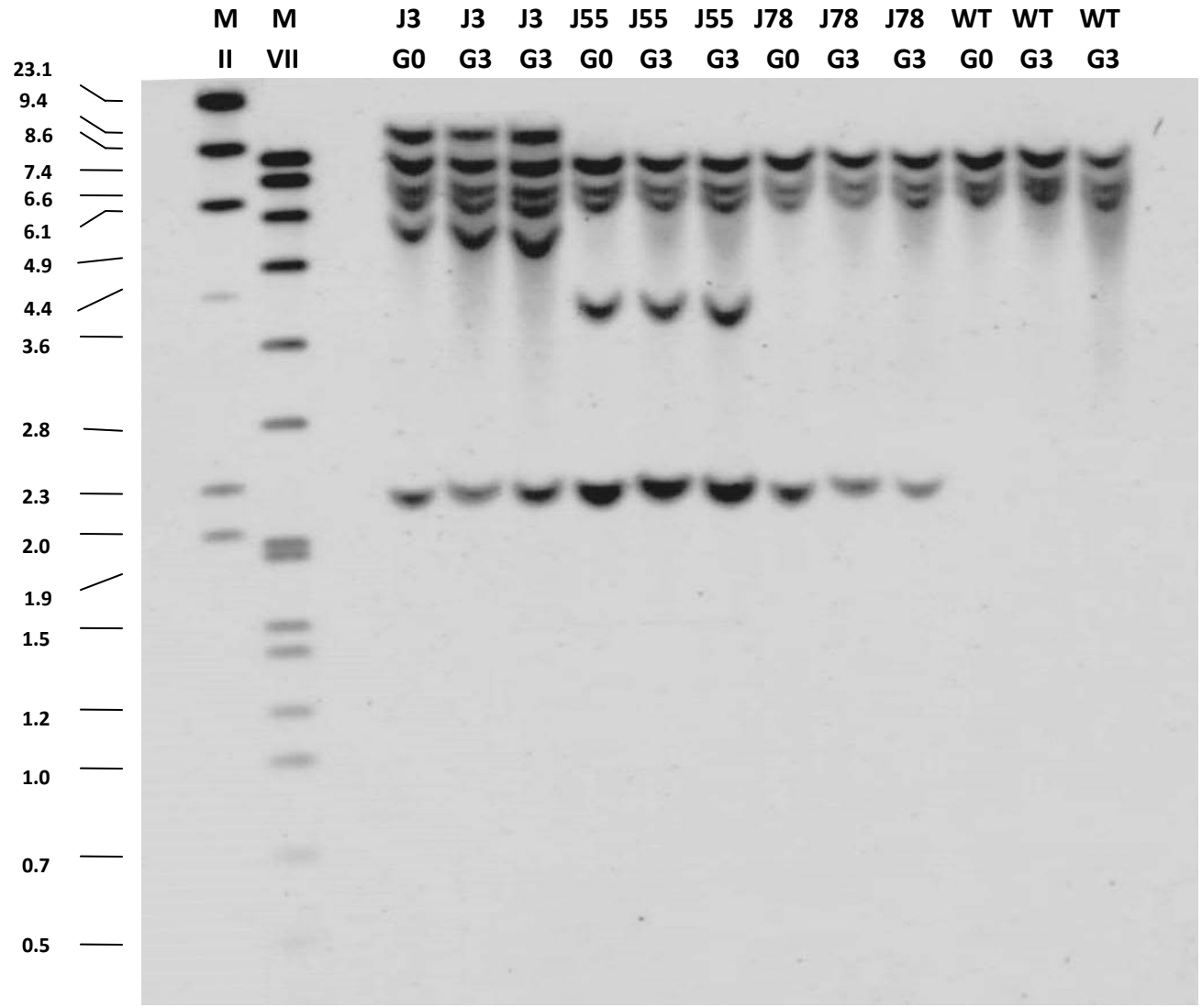
Atlantic untransformed controls (wt), J3, J55, and J78 all show consistency with 7.1 kb, 7.8 kb, 8.6 kb size bands in the G0 and G1 plants. J3 and J78 share a band the size of 2.3 kb in both the G0 and the G1 plants. Additionally, J3 has 5.8 kb and ~12 kb bands in the G0 and G1 plants. J55 has bands of 2.3 kb and 4.1 kb in the G0 and G1 plants.

Figure 16. Atlantic J3, J55, J78: DNA Insert Stability in G2 Samples as Visualized with a GBS Probe



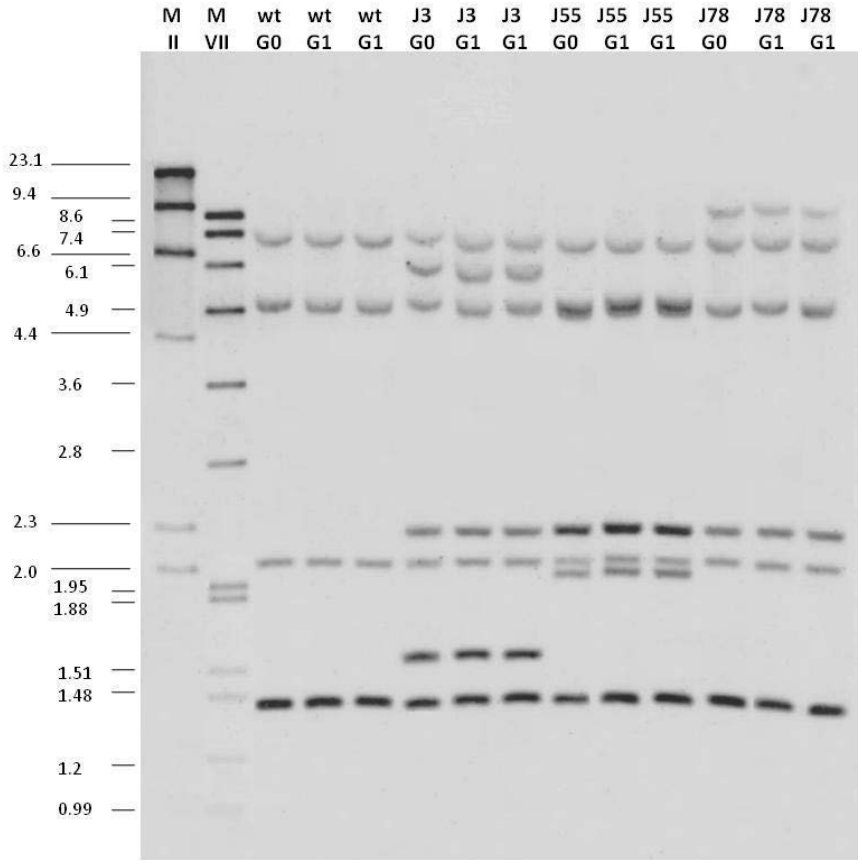
Atlantic untransformed controls (wt), J3, J55, and J78 all show consistency with 7.1 kb, 7.8 kb, 8.6 kb size bands in the G0 and G2 plants. J3 and J78 share a band the size of 2.3 kb in both the G0 and the G2 plants. Additionally, J3 has 5.8 kb and ~12 kb bands in the G0 and G2 plants. J55 has bands of 2.3 kb and 4.1 kb in the G0 and G2 plants.

Figure 17. Atlantic J3, J55, J78: DNA Insert Stability in G3 Samples as Visualized with a GBS Probe[1]



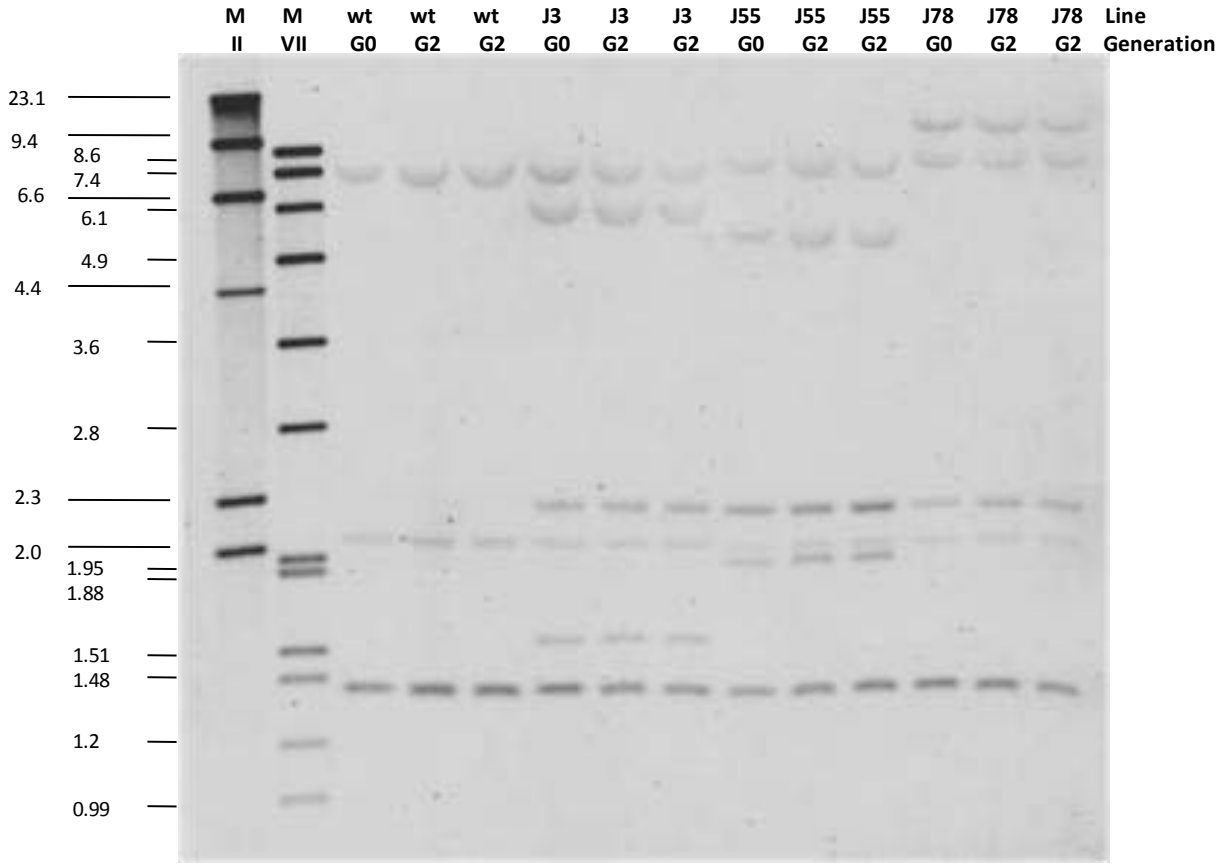
[1]Tubers were unavailable for this test so leaves from third generation plants were used.
Atlantic untransformed controls (wt), J3, J55, and J78 all show consistency with 7.1 kb, 7.8 kb, 8.6 kb size bands in the G0 and G3 plants. J3 and J78 share a band the size of 2.3 kb in both the G0 and the G3 plants. Additionally, J3 has 5.8 kb and ~12 kb bands in the G0 and G3 plants. J55 has bands of 2.3 kb and 4.1 kb in the G0 and G3 plants.

Figure 18. Atlantic J3, J55, J78: DNA Insert Stability in G0 and G1 Samples as Visualized with an AGP Probe



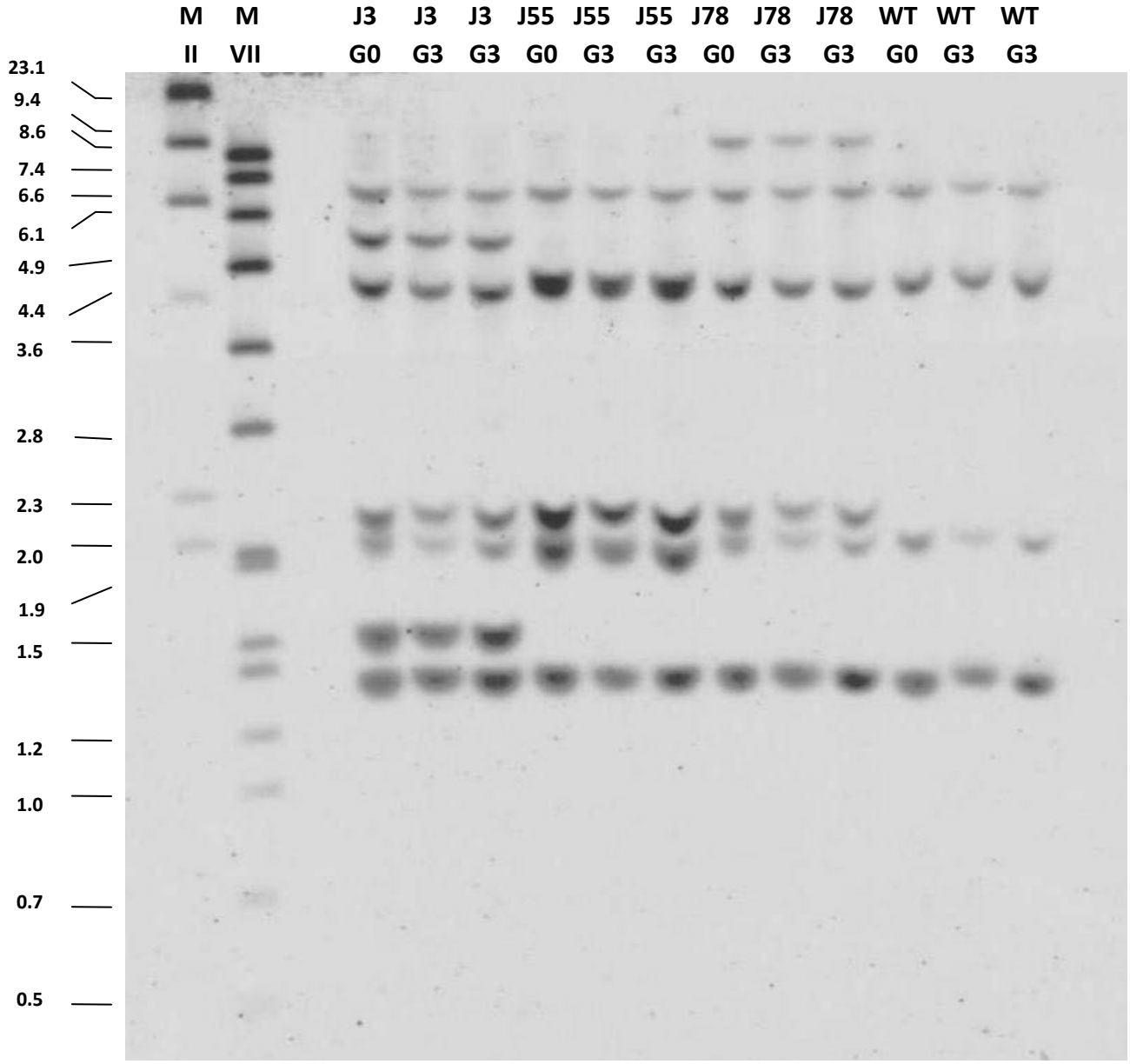
Atlantic untransformed controls (wt), J3, J55, and J78 all show consistency with 1.4 kb, 2.0 kb, 5.0 kb, and 7.2 kb size bands in the G0 and G1 plants. J3 also has bands at 1.6 and 5.7 kb in G0 and G1 plants. J3 and J78 share a band the size of 2.3 kb in both the G0 and the G1 plants. Additionally, J55 has bands the size of 1.95, 2.3, and 4.8 kb in G0 and G1 plants. J78 has a band the size of ~9 kb in G0 and G1 plants.

Figure 19. Atlantic J3, J55, J78: DNA Insert Stability in G2 Samples as Visualized with an AGP Probe



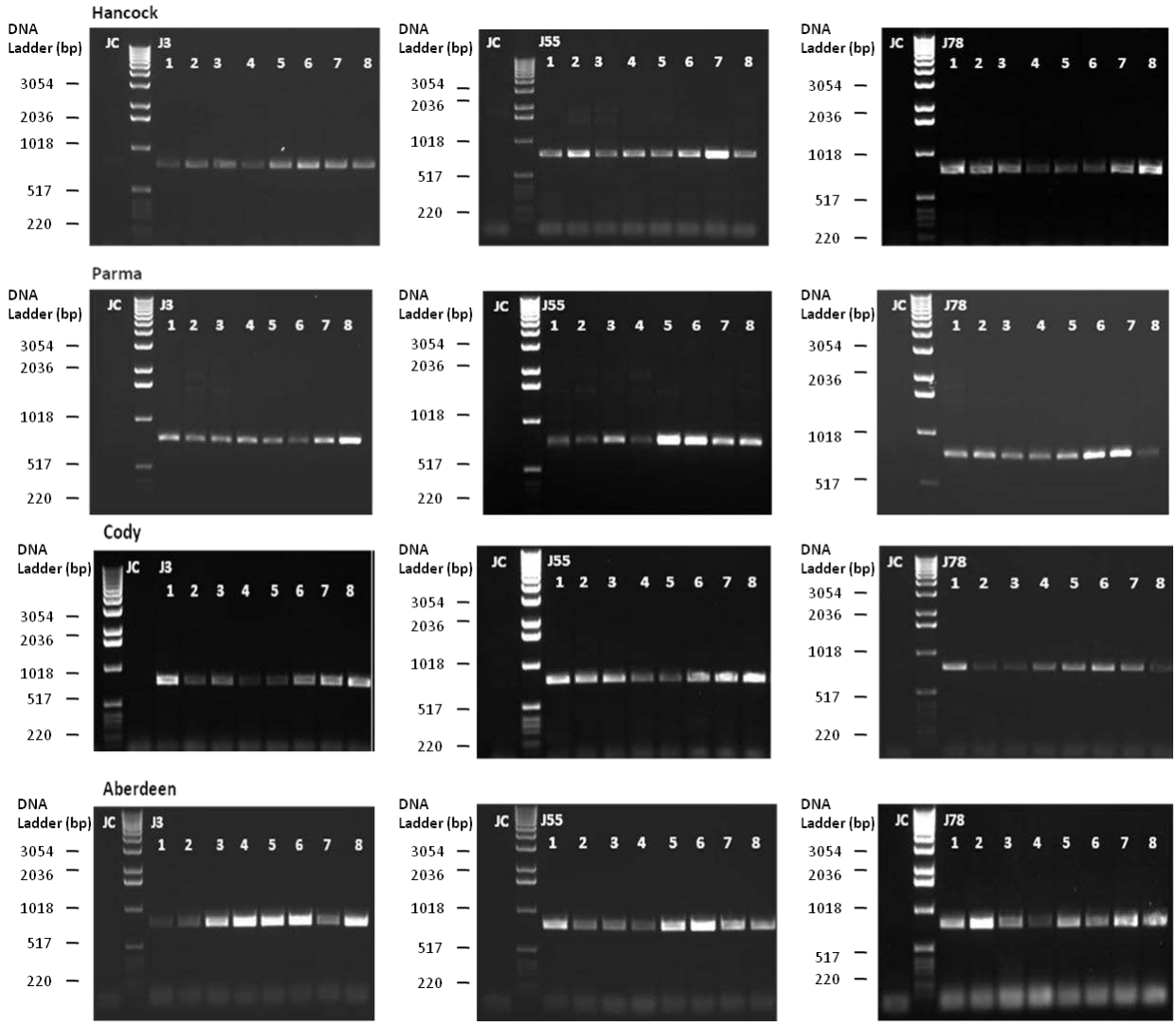
Atlantic untransformed controls (wt), J3, J55, and J78 all show consistency with 1.4 kb, 2.0 kb, 5.0 kb, and 7.2 kb size bands in the G0 and G2 plants. J3 also has bands at 1.6 and 5.7 kb in G0 and G2 plants. J3 and J78 share a band the size of 2.3 kb in both the G0 and the G2 plants. Additionally, J55 has bands the size of 1.95, 2.3, and 4.8 kb in G0 and G2 plants. J78 has a band the size of ~9 kb in G0 and G2 plants.

Figure 20. Atlantic J3, J55, J78: DNA Insert Stability in G3 Samples as Visualized with an AGP Probe[1]



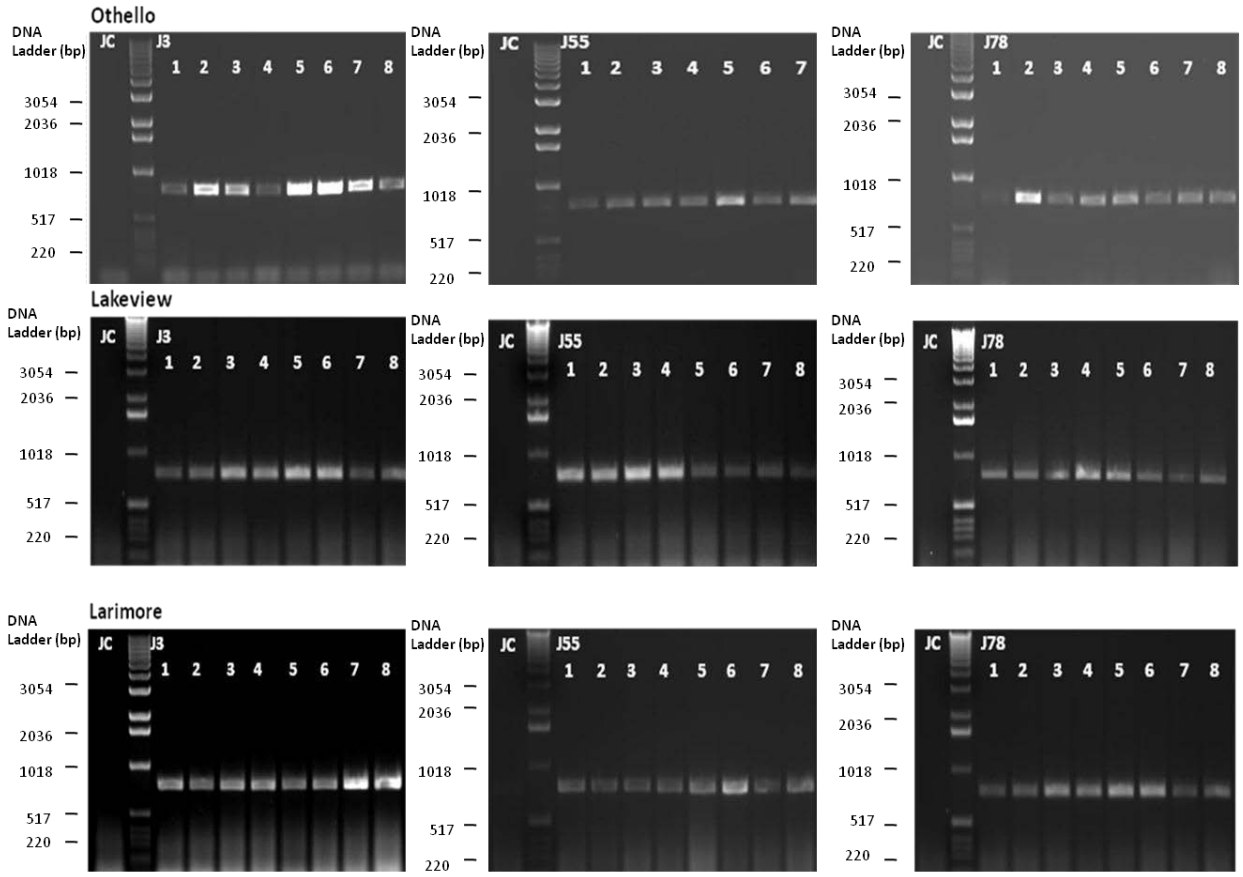
[1]Tubers were unavailable for this test so leaves from third generation plants were used.
Atlantic untransformed controls (wt), J3, J55, and J78 all show consistency with 1.4 kb, 2.0 kb, 5.0 kb, and 7.2 kb size bands in the G0 and G2 plants. J3 also has bands at 1.6 and 5.7 kb in G0 and G2 plants. J3 and J78 share a band the size of 2.2 kb in both the G0 and the G2 plants. Additionally, J55 has bands the size of 1.95, 2.3, and 4.8 kb in G0 and G2 plants. J78 has a band the size of ~9 kb in G0 and G2 plants.

Figure 21. PCR-Based Confirmation of Insert Stability in G2 Tubers of Events J3, J55 and J78



The first lane of each gel contains Atlantic controls labeled JC, followed by a 1 kb DNA ladder (Invitrogen). The subsequent 8 lanes contain DNA from non-systematically chosen G2 tubers from Hancock, Parma, Cody, and Aberdeen. Headings above each gel correspond to the site location where the tubers were grown. A consistent band of 788 bp of DNA insert was amplified from the G2 tubers, but not controls, indicating stable integration of the DNA insert.

Figure 22. PCR-Based Confirmation of Insert Stability in G2 Tubers of Events J3, J55 and J78



The first lane of each gel contains Atlantic controls labeled JC, followed by a 1 kb DNA ladder (Invitrogen). The subsequent 8 lanes contain DNA from non-systematically chosen G2 tubers from Othello, Lakeview, and Larimore. Headings above each gel correspond to the site location where the tubers were grown. A consistent band of 788 bp of DNA insert was amplified from the G2 tubers, but not controls, indicating stable integration of the DNA insert.

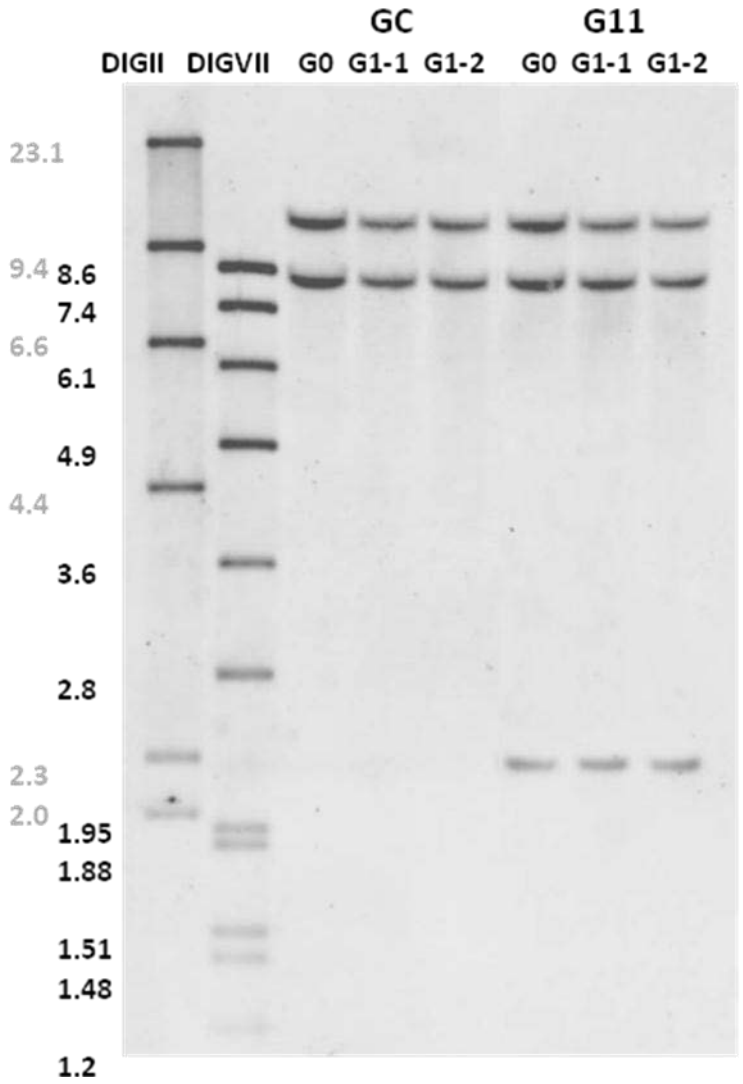
DNA Insert Stability in Event G11

Hybridization of DNA of the G variety and G11 plants visualizes two common bands with the GBS probe (8.2 and ~12 kb) and four with the AGP probe (1.45, 2.1, 5.3, and 8.6 kb) in all lanes. These bands are indicative of DNA fragments of the unmodified genome. One additional internal DNA insert band of 2.2 kb that hybridizes with both the GBS and AGP probes indicates that the insert of the original transformant (G0) remained stable into the first, second, and third vegetative generations G1,G2, and G3 (**Figures 23, 24, 25, 26, 27, and 28**). An additional 5.2 kb band only hybridizing with the AGP probe represented a DNA insert junction fragment and confirmed the insert stability.

Additional evidence for DNA insert stability in event G11 was obtained with the catechol assay for Ppo activity. As shown in **Figure 29**, all event tubers remained uncolored, whereas the untransformed control material developed a deep brown color. This demonstrated that the G11 event had retained their ability to silence the *Ppo5* gene in tubers, after two years of field trials. Preparation of the field trial material had taken the events through repeated tissue culture and vegetative generations without affecting the stability of the inserted genetic material.

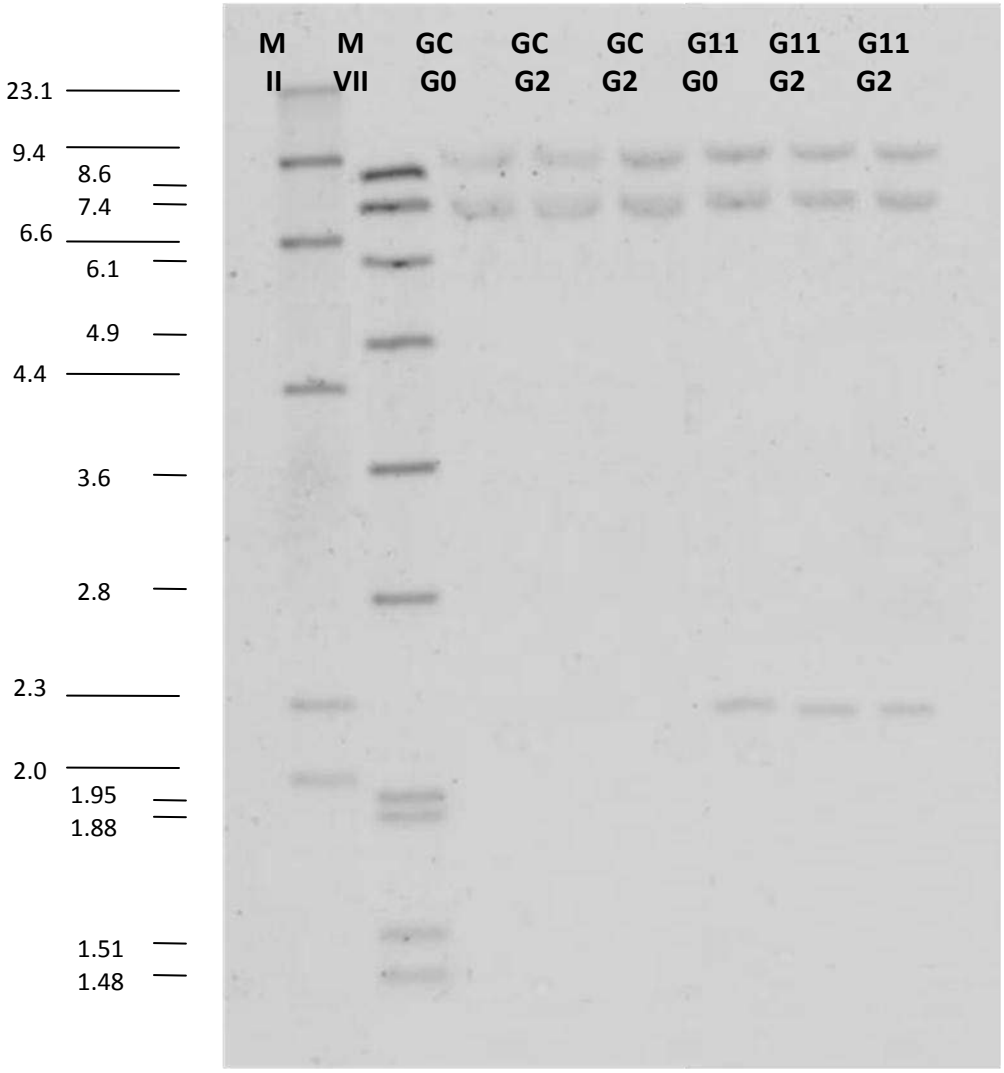
Overall, hyridization (**Figures 23, 24, 25, 26, 27, and 28**) with the GBS and AGP probes shows that event G11 had consistent bands over multiple generations by comparing samples of G0 with G1, G2 and G3. Also, the visual assay for PPO with catechol (**Figure 29**) confirmed stability of *Ppo5* gene silencing from the initial generation through G2.

Figure 23. G11: DNA Insert Stability in G0 and G1 Samples Visualized with a GBS Probe[1]



[1]GC = control G; G1-1 and G1-2 refer to leaves from 2 independent G1 plants
G variety untransformed controls (wt) and G11 show consistency with 8.2 kb and ~12 kb size bands in the G0 and G1 plants. In addition G11 has an additional band of 2.2 kb in both the G0 and G1 plants.

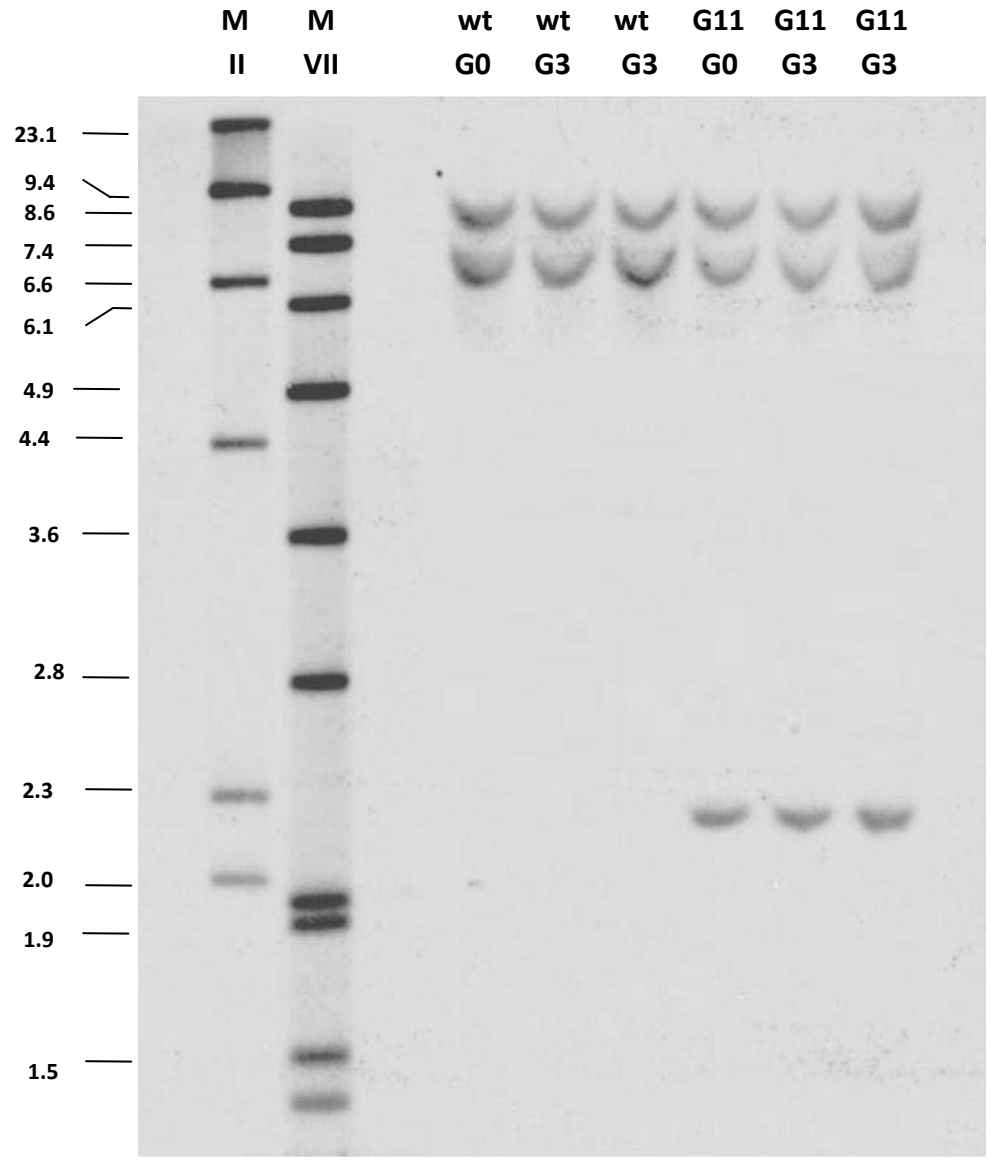
Figure 24. G11: DNA Insert Stability in G2 Samples as Visualized with a GBS Probe[1]



[1]GC = G Control

G variety untransformed controls (wt) and G11 show consistency with 8.2 kb and ~12 kb size bands in the G0 and G2 plants. In addition G11 has an additional band of 2.2 kb in both the G0 and G2 plants.

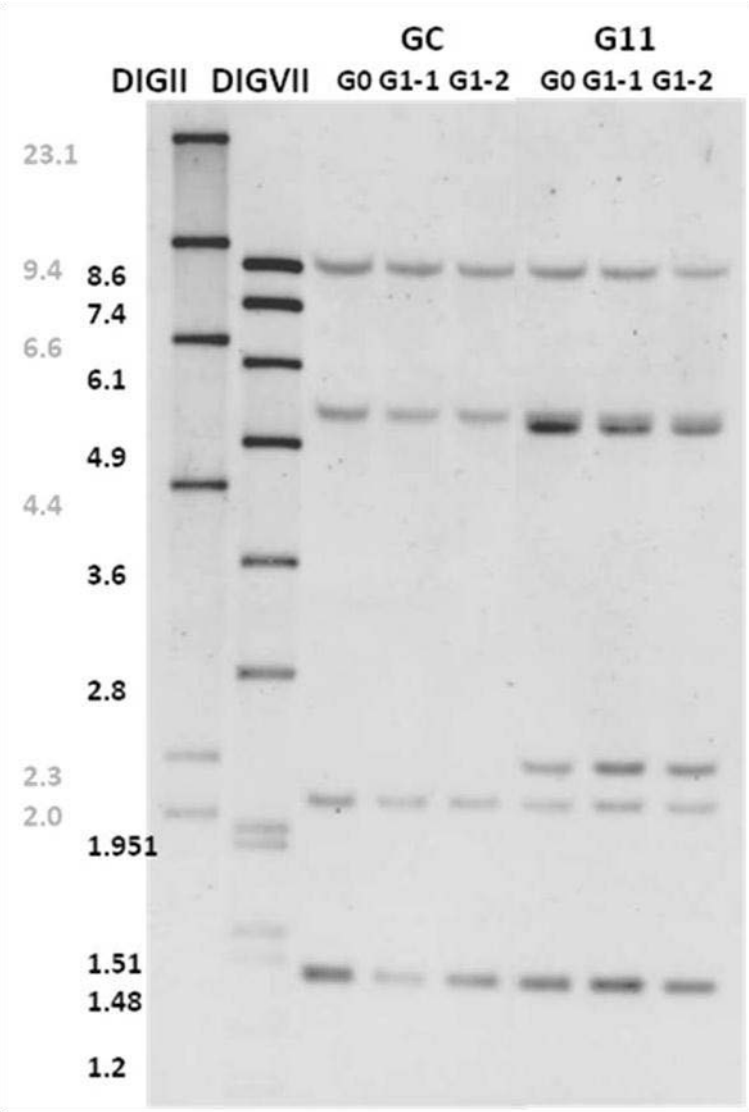
Figure 25. G11: DNA Insert Stability in G3 Samples as Visualized with a GBS Probe



[1]GC = control G

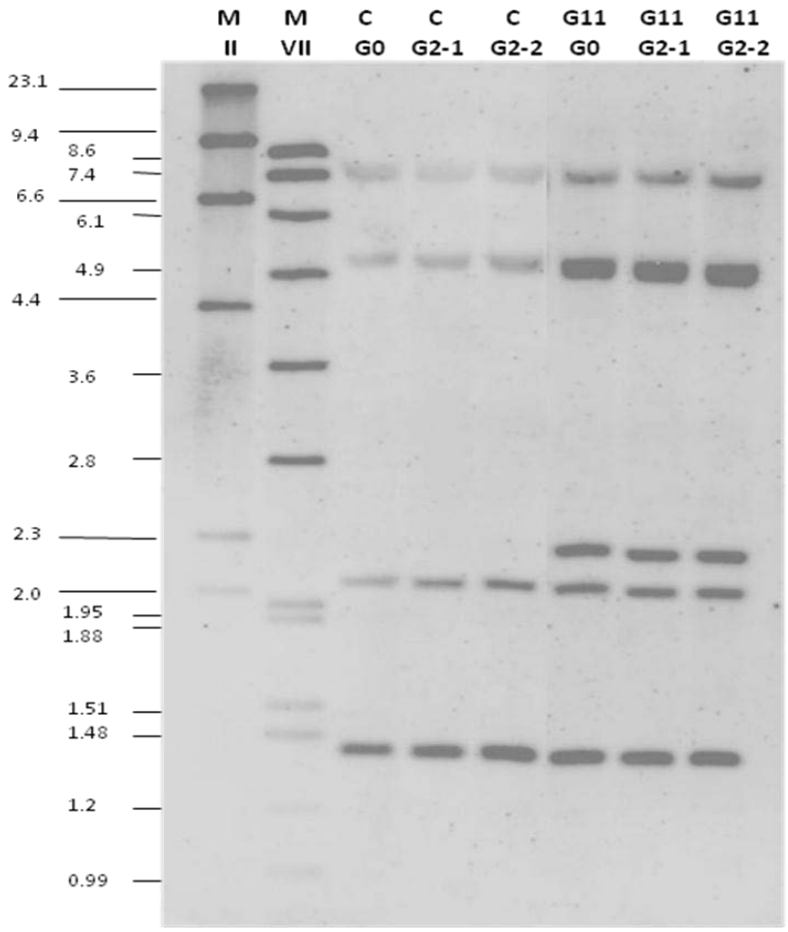
G variety untransformed controls (wt) and G11 show consistency with 8.2 kb and ~12 kb size bands in the G0 and G3 plants. In addition G11 has an additional band of 2.2 kb in both the G0 and G3 plants.

Figure 26. G11: DNA Insert Stability in G0 and G1 Samples Visualized with an AGP Probe[1]



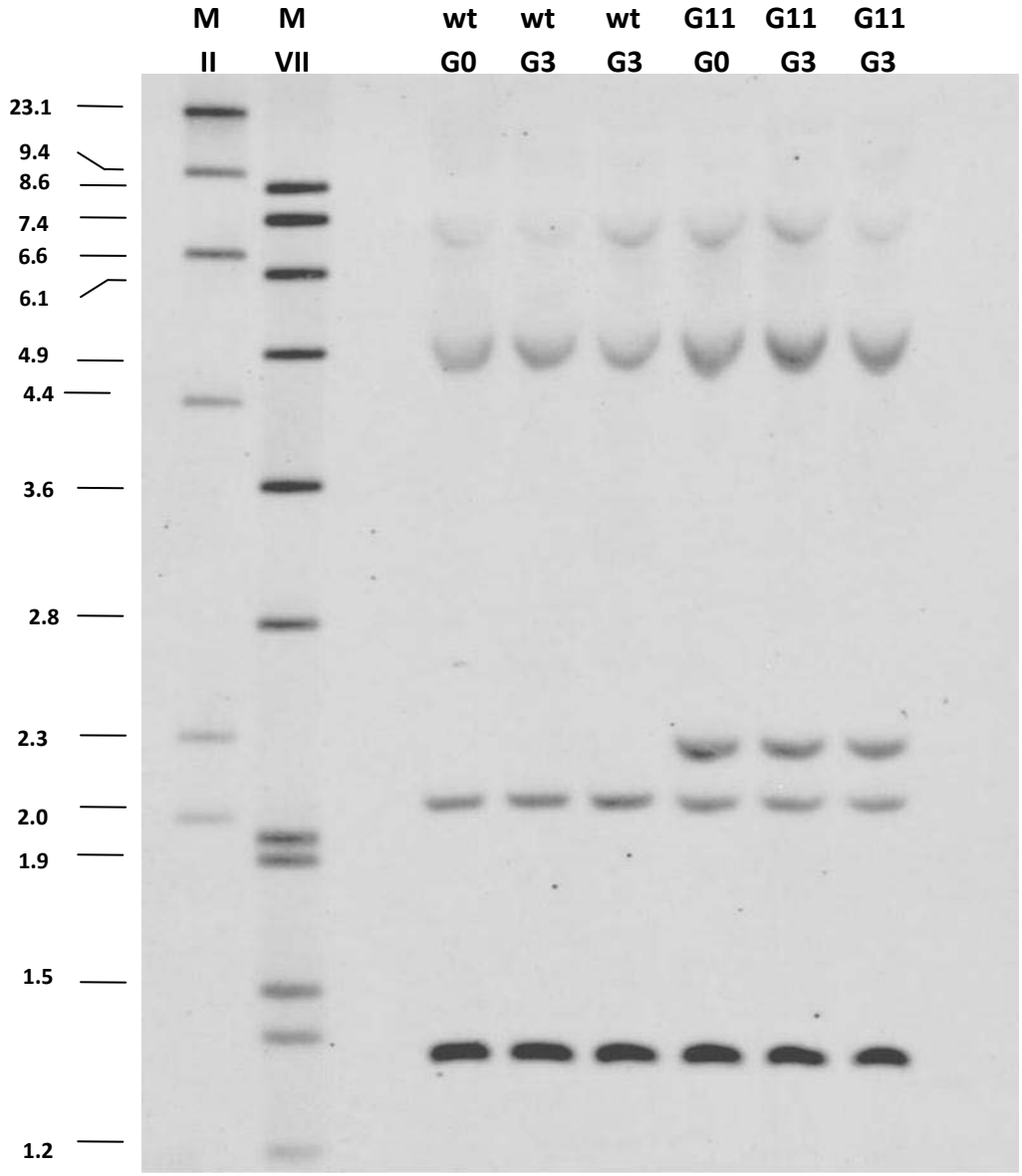
[1]GC = control G, G1-1 and G1-2 refer to samples from 2 independent G1 plants
G variety untransformed controls (wt) and G11 show consistency with 1.45 kb, 2.1 kb, 5.3 kb, and 8.6 kb size bands in the G0 and G1 plants. G11 has two additional size bands of 2.2 kb and 5.2 kb in both the G0 and G1 plants.

Figure 27. G11: DNA Insert Stability in G2 Samples as Visualized with an AGP Probe[1]



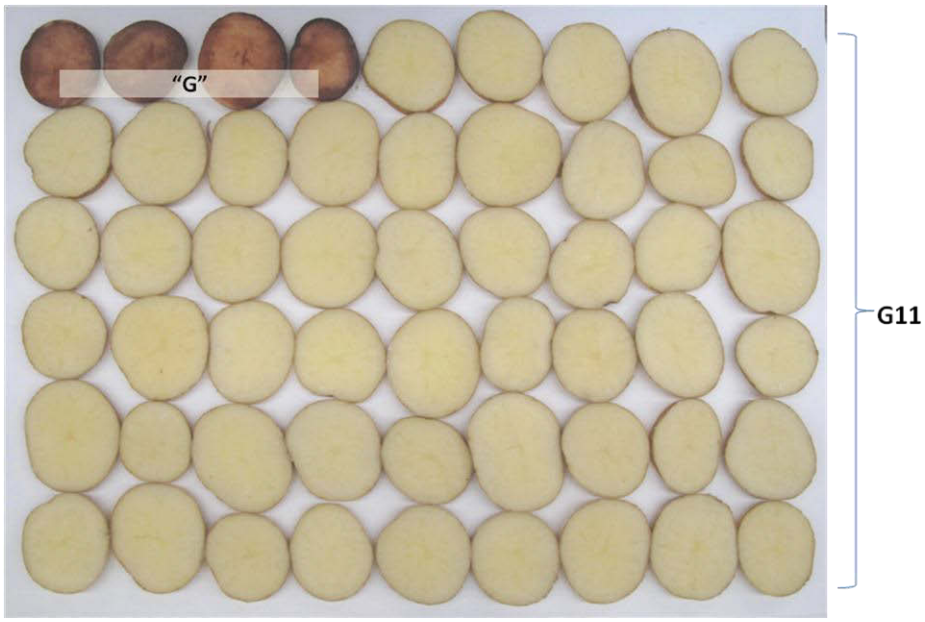
[1]GC = control G
G2-1 and G2-2 refer to samples from 2 independent G2 plants
G variety untransformed controls (wt) and G11 show consistency with 1.45 kb, 2.1 kb, 5.3 kb, and 8.6 kb size bands in the G0 and G2 plants. G11 has two additional size bands of 2.2 kb and 5.2 kb in both the G0 and G2 plants.

Figure 28. G11: DNA Insert Stability in G3 Samples as Visualized with an AGP Probe



G variety untransformed controls (wt) and G11 show consistency with 1.45 kb, 2.1 kb, 5.3 kb, and 8.6 kb size bands in the G0 and G3 plants. G11 has two additional size bands of 2.2 kb and 5.2 kb in both the G0 and G3 plants.

Figure 29. Assay for Sustained *Ppo5* Gene Silencing in G2 Tubers of Event G11



DNA Insert Stability in Events H37 and H50

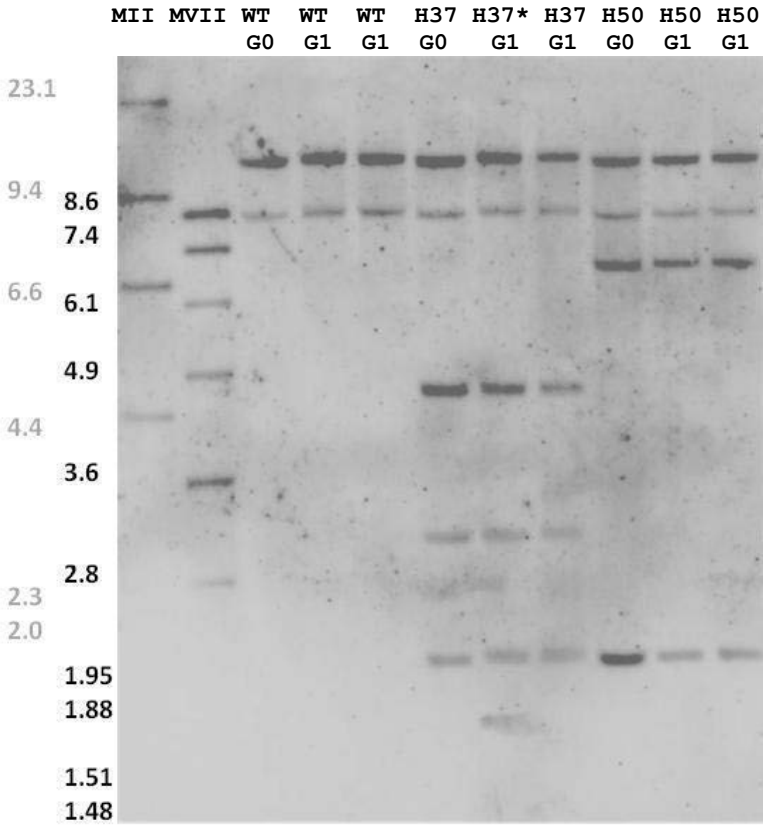
Hybridization of DNA of the H variety as well as H37 and H50 plants visualizes two common bands with the GBS probe (7.8 and 10.0 kb) and five with the AGP probe (1.4, 2.1, 5.4, 6.7, and 8.0) in all lanes. These bands are indicative of DNA fragments of the unmodified genome. Three additional GBS-hybridizing bands in DNA of H37, one 2.3 kb band indicative of an internal DNA insert fragment and two others representing DNA insert junction fragments (3.0 and 4.5 kb), indicate that the inserts of the original transformants (G0) remained stable into the first, second, and third vegetative generations G1, G2, and G3 (**Figures 30, 31, 32, 33, 34, and 35**). Similarly, there are three additional bands consistently visualized with the AGP probe in H37 DNA (2.0, 3.2 and 3.6 kb).

Event H50 also contains the 2.3 kb internal DNA insert fragment in all generations. Additionally, it carries a single junction fragment hybridizing with the GBS probe (6.5 kb) and two with the AGP probe (1.9 and 3.8 kb), as was expected based on the DNA insert organization of this event.

Additional evidence for DNA insert stability in events H37 and H50 was obtained with the catechol assay for Ppo activity. As shown in **Figures 36 and 37**, all event tubers remained uncolored, compared with the dark-colored untransformed control material. This demonstrated that these events for this variety had retained their ability to silence the *Ppo5* gene in G2 tubers, after two years of field trials. Preparation of the field trial material had taken the events through repeated tissue culture and vegetative generations without affecting the stability of the inserted genetic material.

Overall, hyridization (**Figures 30, 31, 32, 33, 34, and 35**) with the GBS and AGP probes shows that events H37 and H50 had consistent bands over multiple generations by comparing samples of G0 with G1, G2 and G3. Also, the visual assay for PPO with catechol (**Figures 36 and 37**) confirmed stability of *Ppo5* gene silencing from the initial generation through G2.

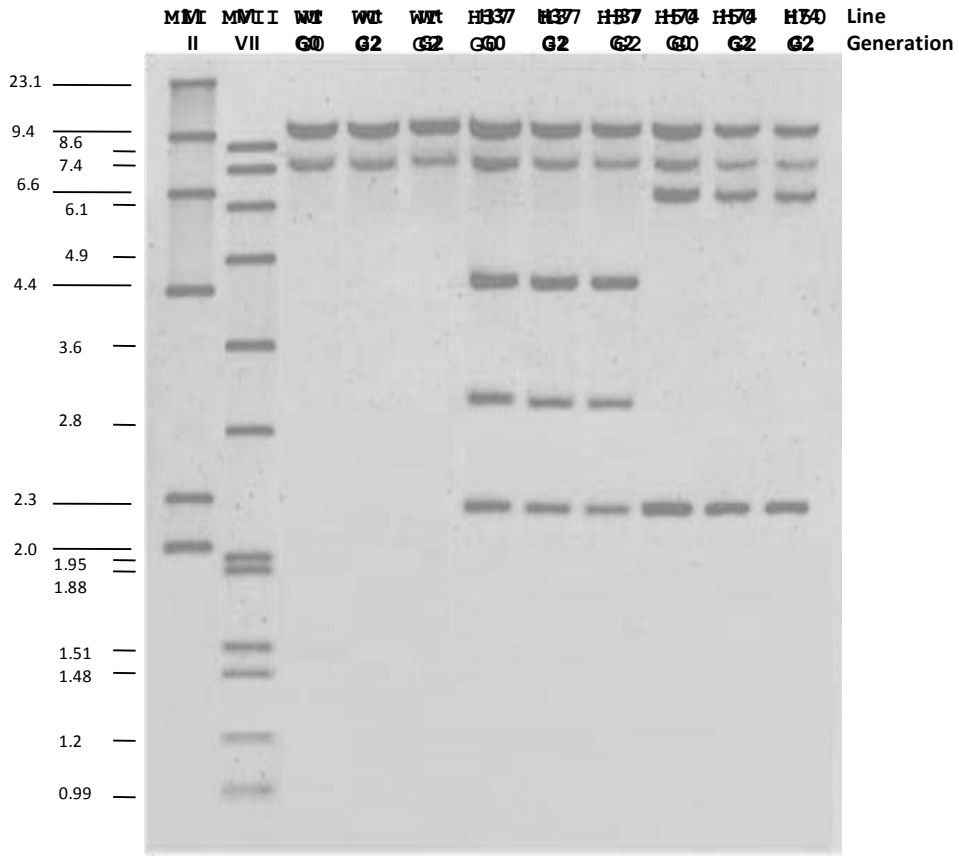
Figure 30. H37 and H50: DNA Insert Stability in G0 and G1 Samples Visualized with a GBS Probe



*Background smear in the lane marked "H37 G1" at 1.7 kb is not a band.

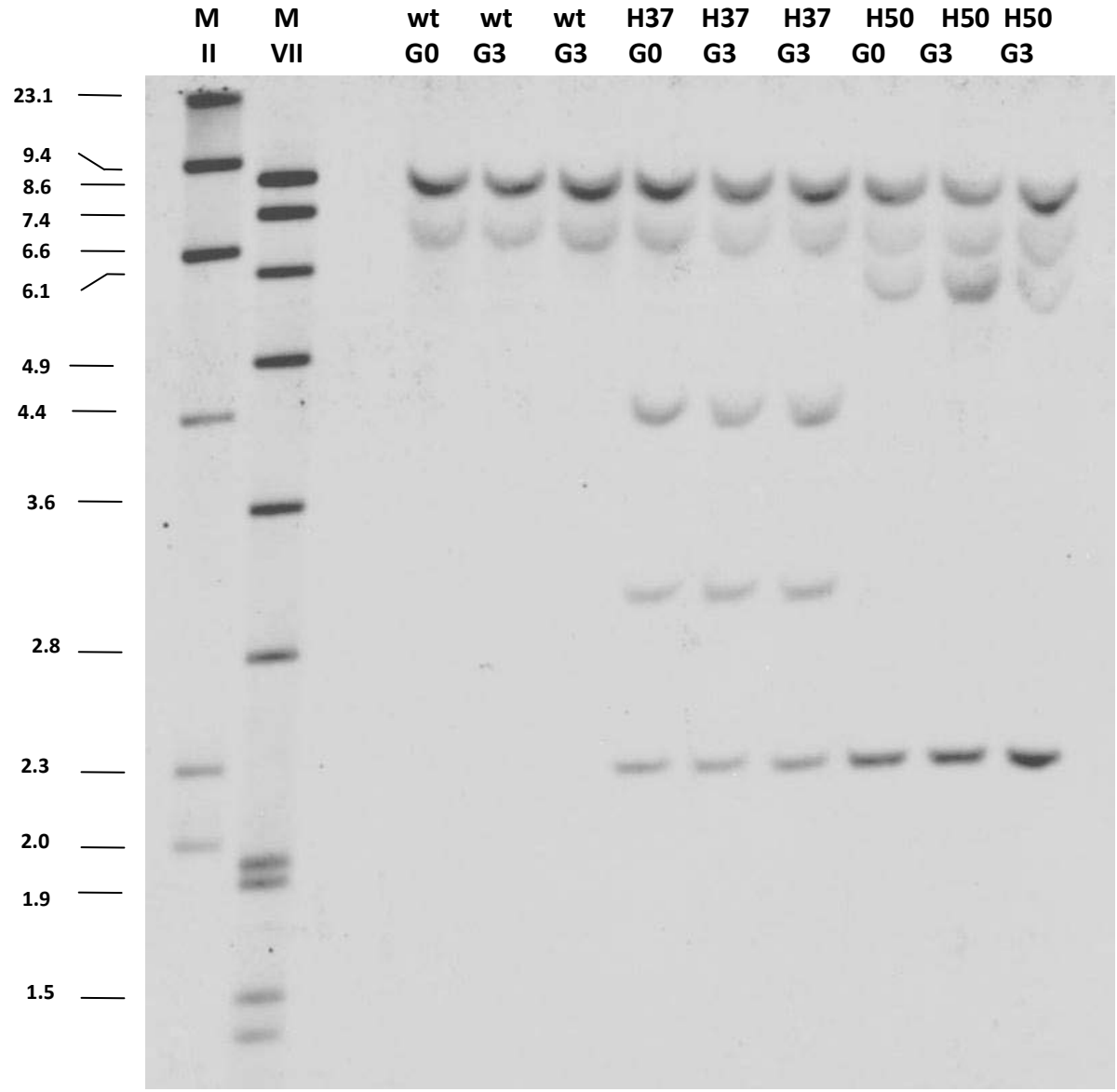
H variety untransformed controls (wt), H37, and H50 show consistency with 7.8 kb and 10.0 kb size bands in the G0 and G1 plants. H37 and H50 have one additional band of 2.3 kb in size in G0 and G1 plants. Additionally, H37 has two bands of 3.0 kb and 4.5 kb in size. H50 has one additional band with a size of 6.5 kb in G0 and G1 plants.

Figure 31. H37 and H50: DNA Insert Stability in G0 and G2 Samples Visualized with a GBS Probe



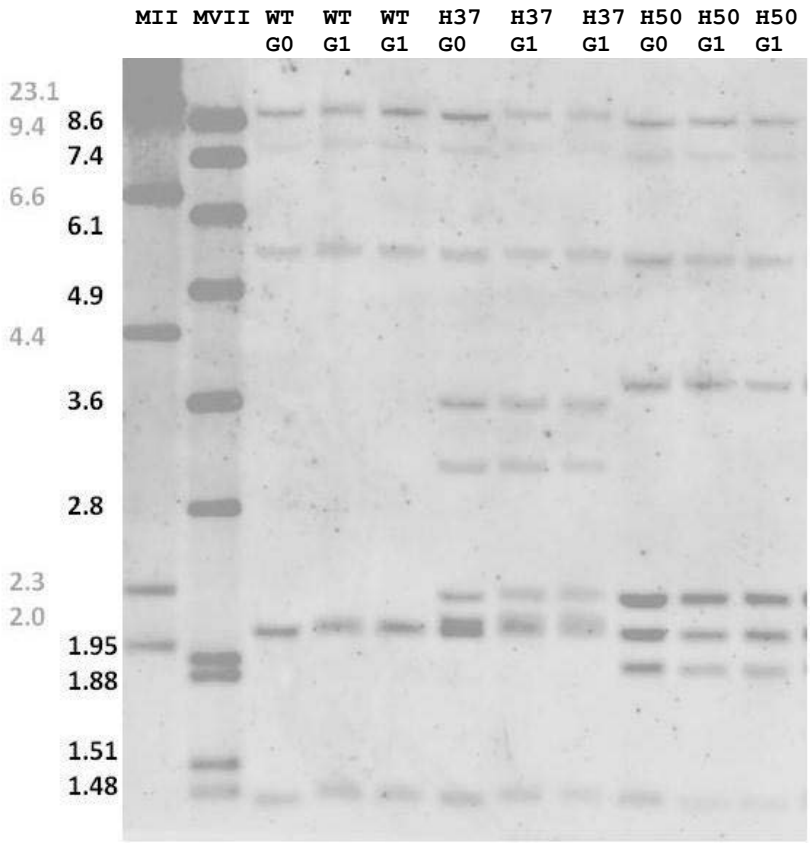
H variety untransformed controls (wt), H37, and H50 show consistency with 7.8 kb and 10.0 kb size bands in the G0 and G2 plants. H37 and H50 have one additional band of 2.3 kb in size in G0 and G2 plants. Additionally, H37 has two bands of 3.0 kb and 4.5 kb in size. H50 has one additional band with a size of 6.5 kb in G0 and G2 plants.

Figure 32. H37 and H50: DNA Insert Stability in G3 Samples as Visualized with a GBS Probe



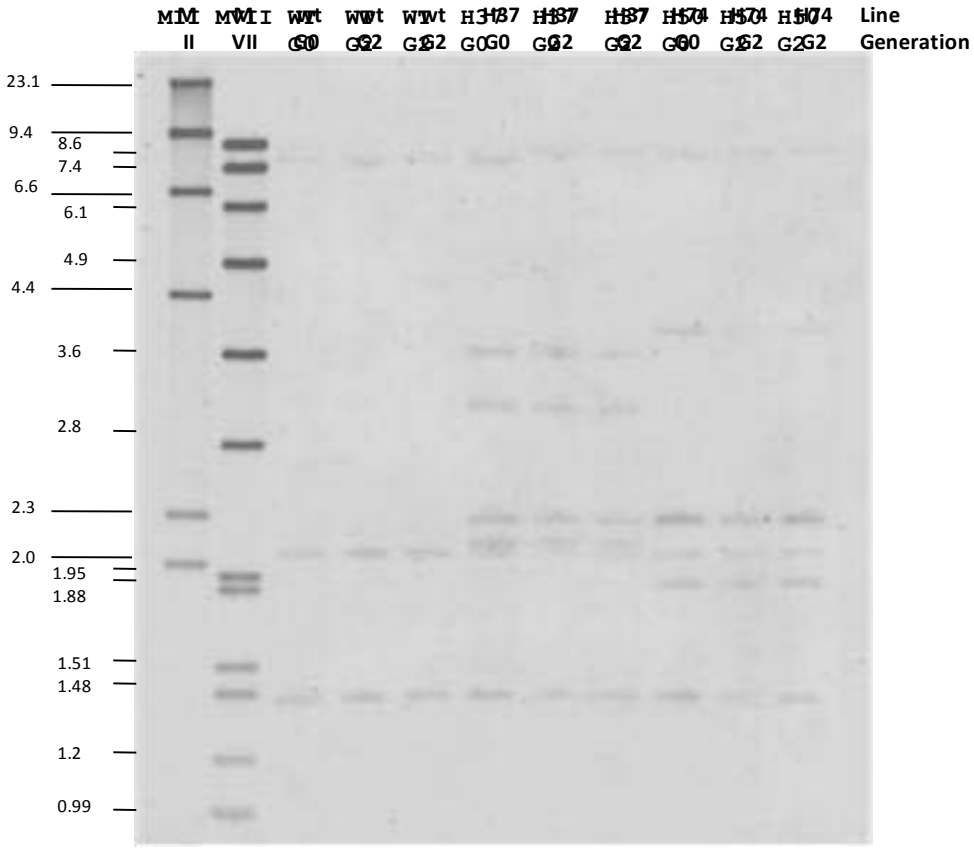
H variety untransformed controls (wt), H37, and H50 show consistency with 7.8 kb and 10.0 kb size bands in the G0 and G3 plants. H37 and H50 have one additional band of 2.3 kb in size in G0 and G3 plants. Additionally, H37 has two bands of 3.0 kb and 4.5 kb in size. H50 has one additional band with a size of 6.5 kb in G0 and G3 plants.

Figure 33. H37 and H50: DNA Insert Stability in G0 and G1 Samples Visualized with an AGP Probe



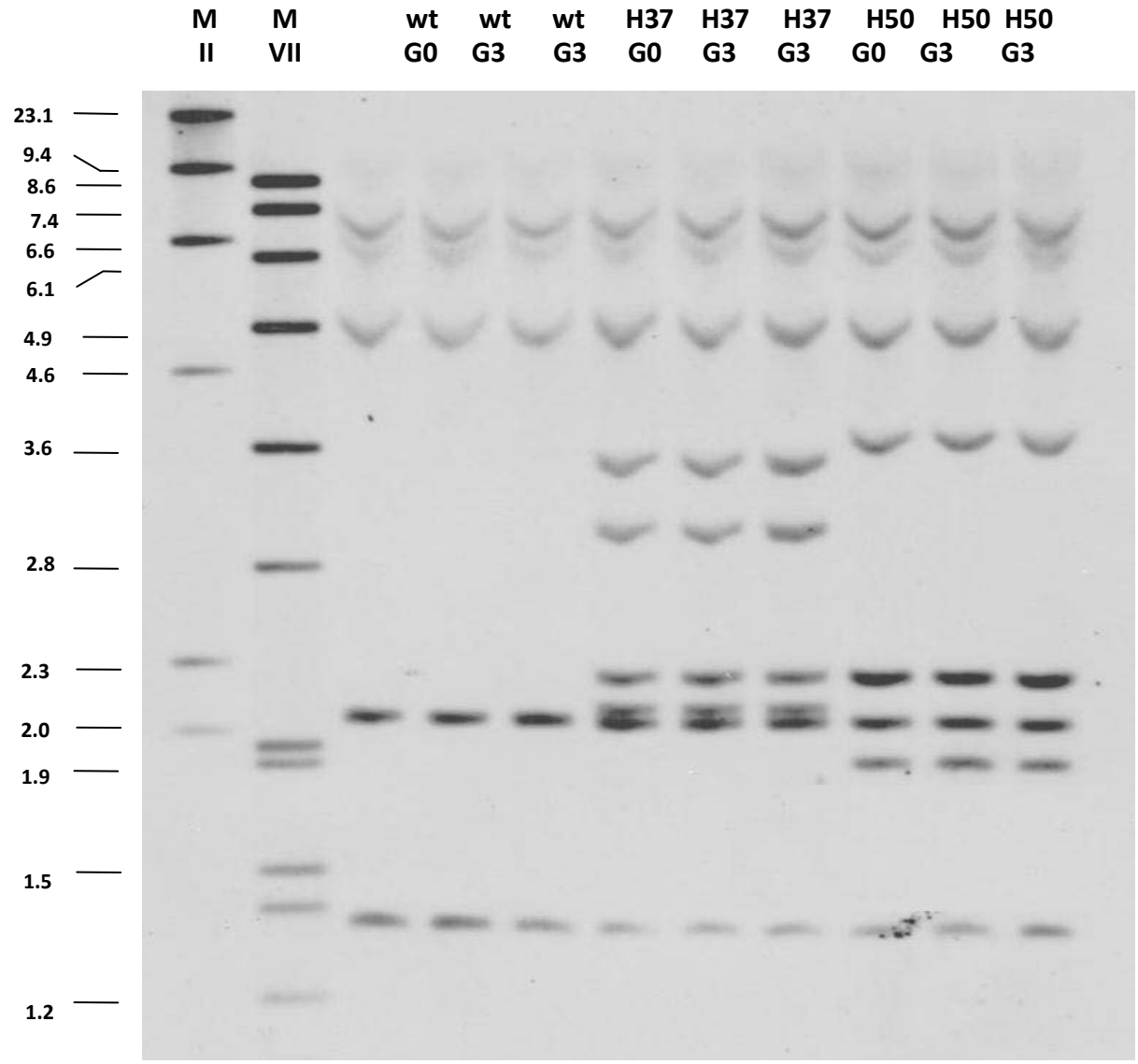
H variety untransformed controls (wt), H37, and H50 show consistency with 1.4 kb, 2.1 kb, 5.4 kb, 6.7 kb, and 8.0 kb size bands in the G0 and G1 plants. H37 contains three additional bands of 2.0 kb, 3.2 kb, and 3.6 kb in size. H50 has two additional bands of 1.9 kb and 3.8 kb in size in G0 and G1 plants.

Figure 34. H37 and H50: DNA Insert Stability in G0 and G2 Samples Visualized with an AGP Probe



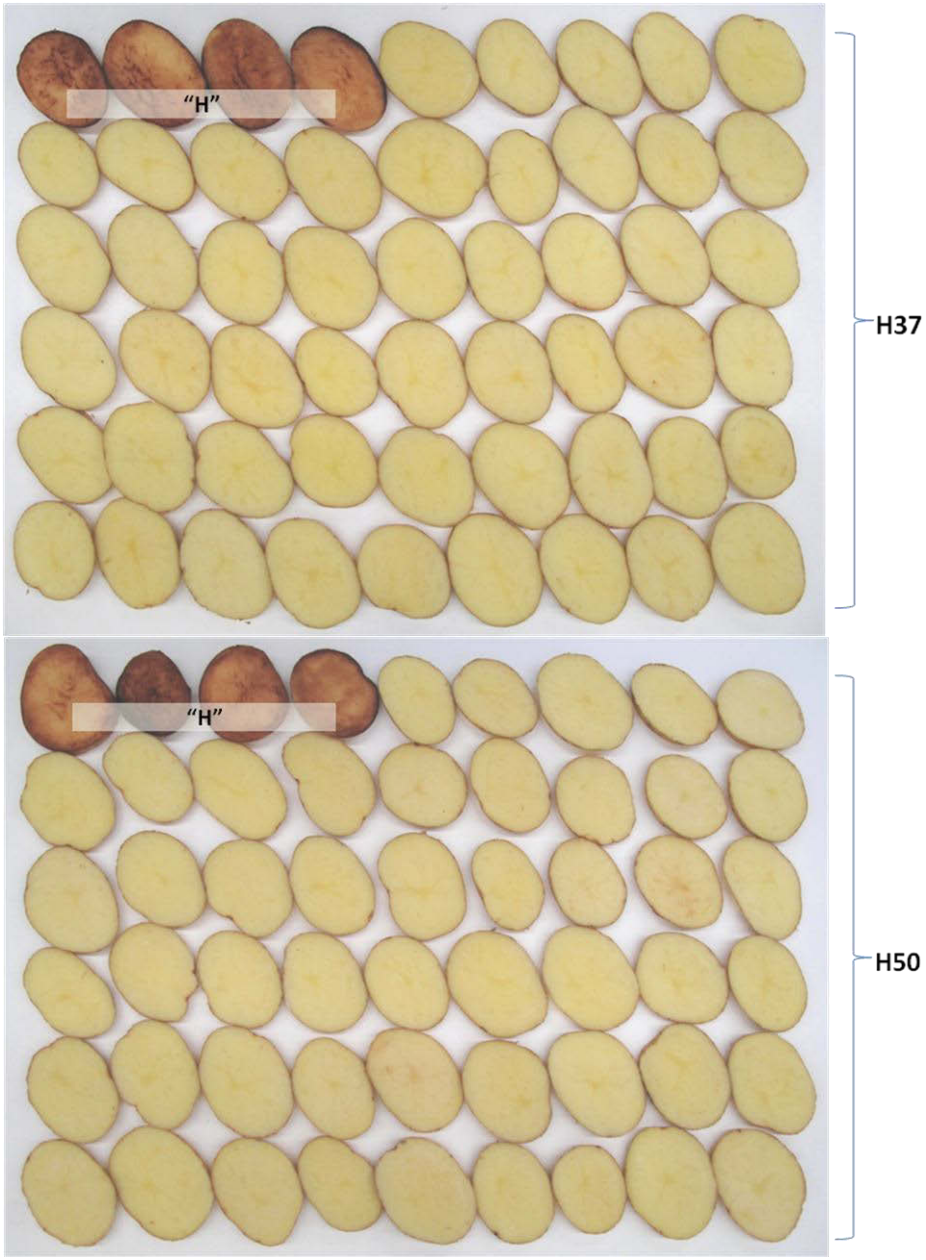
H variety untransformed controls (wt), H37, and H50 show consistency with 1.4 kb, 2.1 kb, 5.4 kb, 6.7 kb, and 8.0 kb size bands in the G0 and G2 plants. H37 contains three additional bands of 2.0 kb, 3.2 kb, and 3.6 kb in size. H50 has two additional bands of 1.9 kb and 3.8 kb in size in G0 and G2 plants.

Figure 35. H37 and H50: DNA Insert Stability in G3 Samples as Visualized with an AGP Probe



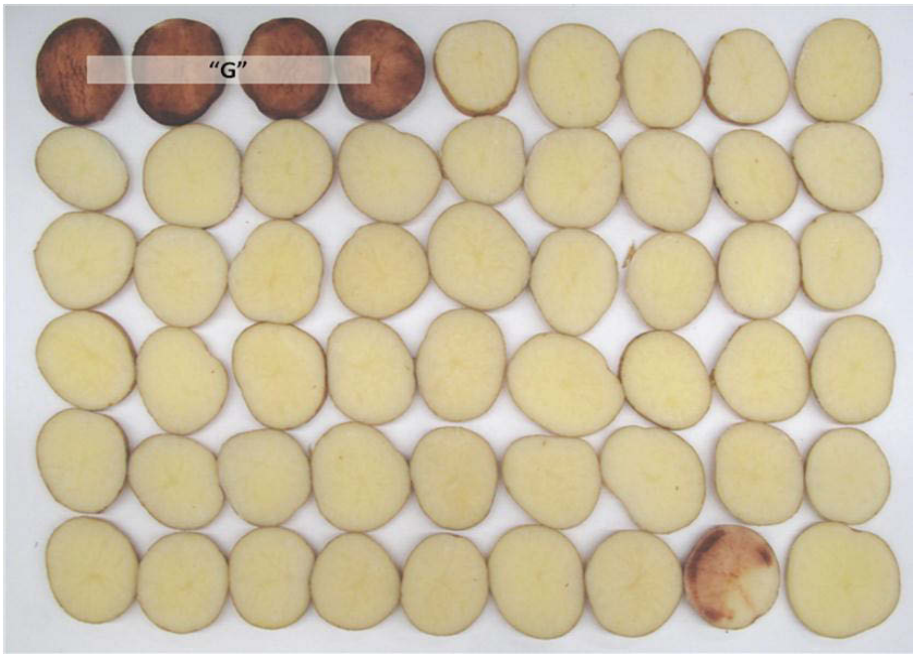
H variety untransformed controls (wt), H37, and H50 show consistency with 1.4 kb, 2.1 kb, 5.4 kb, 6.7 kb, and 8.0 kb size bands in the G0 and G3 plants. H37 contains three additional bands of 2.0 kb, 3.2 kb, and 3.6 kb in size. H50 has two additional bands of 1.9 kb and 3.8 kb in size in G0 and G3 plants.

Figures 36. Assay for Sustained *Ppo5* Gene Silencing in G2 Tubers of Events H37 and H50



It should be mentioned here that this assessment did identify insert instability in another event, which is not part of this petition. The G2 tubers of this event displayed instability at a low frequency (**Figure 37**). Of the 50 tubers tested, one tuber developed some color when subjected to catechol. This color development is probably indicative of a partial reversion to *Ppo5* gene expression. Importantly, this indicates that the visual catechol test is effective for detecting gene instability in potato events transformed with this construct. Given the demonstrated sensitivity of the test to identify low frequencies of instability, it will be used to monitor trait stability in commercial potato seed (except for Atlantic, where the PCR-based stability test will be used).

Figure 37. Assay for *Ppo5* Gene Silencing in G2 Tubers of a G Event That is Not Considered for Deregulation



DISCUSSION

Given the demonstrated DNA insert stability in the 10 vegetatively propagated events described in this petition, it is unlikely for instability to arise during subsequent cycles of vegetative propagation. Continued trait efficacy could be readily confirmed by performing the catechol assay for Ppo activity on non-systematically chosen tubers. This assay is indicative of not only black spot bruise tolerance but also low asparagine formation because *Ppo5* and *Asn1* gene silencing is mediated by the same silencing cassette. In the unlikely case that a specific line would display trait instability, individual tubers would revert back, either partially or fully, to the phenotype of their untransformed counterparts. Such reversion events will not be associated with the expression of phenotypes associated with pests or noxious weeds. For the Atlantic events, the PCR detection assay can be used instead of the catechol assay.

MATERIALS and METHODS

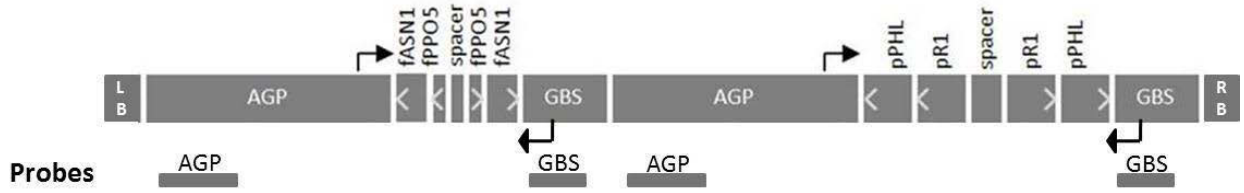
Materials. DNA insert stability was demonstrated in the originally-transformed material (G0) by extracting and evaluating DNA from leaves of plants that had been propagated *in vitro* and never planted in soil. For generation-1 (G1) analyses, two propagated plants from each event and one plant from each control were planted in the greenhouse; one of the tubers harvested from each plant was planted to obtain leaves from G1 plants that were used to isolate DNA and evaluate the G1 generation. Tubers from this generation were planted again, and leaves of the resulting G2 plants allowed a characterization of that generation. For event F37, G2 tubers rather than leaves were used for the analyses. For all G3 samples, the genomic DNA was extracted from tubers.

In summary, all G0, G1, and G2 DNA was from leaves except that G2 material from F37 was from tubers, and G3 material all came from tubers. Extracted DNA from leaves or tubers were digested with *Eco*RV and hybridized with two probes (GBS and AGP).

DNA Isolation. For plant DNA isolation, 1.0 g of young potato leaves was ground into a fine powder in a mortar using 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 until ready to process. 10 ml extraction buffer (0.35 M Sorbitol, 0.1 M Tris-HCl, pH8.0, 0.05M EDTA) was added to, and mixed with, the powder, and the resulting suspension was centrifuged at 3,000 rpm for 15 min at room temperature. The pellet was thoroughly re-suspended in 2 ml extraction buffer containing 200 µg RNase A. After incubating the resuspended DNA at 65°C for 20 min with 2 ml of nuclear lysis buffer (0.2 M Tris-HCl pH7.5, 0.005 M EDTA pH 8.0 and 20 mg/ml CTAB Hexadecyl Trimetyl Ammonium Bromide) and 800 µl of 5% Sarcosyl, it was mixed with an equal volume of chloroform: isoamyl alcohol (24:1), vortexed for about 1 min, and centrifuged at 3000 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 a spectrophotometer at an OD of 260 nm, whereas 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. In some cases, an alternative method was used to isolate DNA. The ground tissue from 0.7 g young leaves was mixed with 7 ml CTAB buffer (2% CTAB, 1.4 M NaCl, 0.1 M pH 8.0 Tris-HCl, 20 mM pH8.0 EDTA, 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 3000 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 dried in air and dissolved in TE buffer. The quality and concentration of DNA was determined spectrophotometrically and by running a sample on a 0.8% agarose gel. It should be mentioned here that control and event DNA were always extracted with the same method for a specific comparison. For event F37, G2 tubers rather than leaves were used for DNA extraction using the same protocol.

DNA Gel (Southern) Blot Analysis. The probes used in the Southern blot analysis are represented in **Figure 38**. 10 µg of 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, pH5.3 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.

Figure 38. Probes Used for the Southern Analysis



Membrane Preparation. Digested DNA was loaded on a large 0.7% agarose gel (170 ml) containing 0.5X TBE buffer and 3-5 µl ethidium bromide (10 mg/ml) and run at 25 volts for 18 hrs. The gel was photographed using a gel documentation system from Alpha Innotech (Santa Clara, California), and then depurinated by submerging it into 0.25 N HCl for 20 min. After subsequent denaturing in 0.5 M NaOH /1.5 M NaCl for 2 X 15 min and neutralization in 1.5 M NaCl and 0.5 M Tris-HCl, pH7.5, for 2X15 min on a shaker at room temperature and 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 (Sambrook and Russell 2001).

PCR-based stability test. For Atlantic, insert stability was evaluated with PCR using tuber-extracted DNA as template and the primer set HD121F (5'-CCTCTCGTAAATTCCGACAAA, at position 2113-2133 of the DNA insert, which is the 3' end of AGP promoter) and HD121R (5'-TCTCAACTTGCGAACCTGAC, at position 2882-2901 of the spacer in the first inverted repeat) to amplify a 789-bp DNA insert fragment containing part of the *Asn1/Ppo5* gene. **Figure 39** below shows the location of the amplified fragment on the DNA insert.

Figure 39. PCR Primer Positions for Confirmation of Stability in Atlantic Events

Catechol assay for Ppo activity. The second stability assessment of the events was based on their black spot bruise susceptibility. The events including Ranger Russet and Russet Burbank were planted in plots with three replications in the spring for the 2nd year field trials (2010) in Parma, Larimore, Aberdeen, and Othello (**Table 1)**. They were grown and harvested using standard commercial production practices. For each event, twelve or thirteen tubers from each site (four or five from each rep) for a total of 50 G2-tubers from all four sites were non-systematically selected from harvested tubers (see the following **Table 1**). Similarly, G and H plants were planted in Rhinelander, WI and 50 G2-tubers were non-systematically selected from harvested tubers (**Table 1).** One slice of each tuber was then assayed for the absence of the functional PPO enzyme by adding 1.0 mL of 25 mM catechol in 50 mM MOPS buffer, pH 6.5, onto each freshly cut tuber slice surface and reviewing for color development.

Table 1. Number of Tubers from Each Location Assayed for PPO Activity with Catechol

Location	Event F10	Event F37	Event E12	Event E24	Event G11	Event H37	Event H50
Parma, ID	13	13	13	13	*	*	*
Larimore, ND	12	12	12	12	*	*	*
Aberdeen, ID	13	12	12	12	*	*	*
Othello, WA	12	13	13	13	*	*	*
Rhinelander, WI	*	*	*	*	50	50	50
Total Number of Tubers	50	50	50	50	50	50	50

* None from this location

CONCLUSIONS

Overall Conclusion: Our data provides evidence that the genetic insertions remain stable, which was confirmed in the genotype and phenotype of the 10 events that are the subject of this submission, meaning that their inserts are stable in the original transformants as well as in G1, G2, and G3 plant materials. Because potato is a vegetatively propagated crop, material from tissue culture and tubers were used in stability testing.

Ranger Russet Events F10 and F37: Hybridization with the GBS and AGP probes shows that events F10 and F37 had consistent bands over multiple generations by comparing samples of G0 with G1, G2 and G3. Also, the visual assay for PPO with catechol confirmed stability of *Ppo5* gene silencing from the initial generation through G2.

Russet Burbank Events E12 and E24: Hybridization with the GBS and AGP probes shows that events E12 and E24 had consistent bands over multiple generations by comparing samples of G0 with G1, G2 and G3. Also, the visual assay for PPO with catechol confirmed stability of *Ppo5* gene silencing from the initial generation through G2.

Atlantic Events J3, J55, and J78: Hybridization with the GBS and AGP probes shows that events J3, J55, and J78 had consistent bands over multiple generations by comparing samples of G0 with G1, G2 and G3. Also, PCR testing showed amplification of the same 788 bp DNA insert fragment in all G2 events, that was not found in the control Atlantic samples, indicating that the DNA insert was stable through at least the second generation.

G Event G11: Hybridization with the GBS and AGP probes shows that event G11 had consistent bands over multiple generations by comparing samples of G0 with G1, G2 and G3. Also, the visual assay for PPO with catechol confirmed stability of *Ppo5* gene silencing from the initial generation through G2.

H Events H37 and H50: Hybridization with the GBS and AGP probes shows that events H37 and H50 had consistent bands over multiple generations by comparing samples of G0 with G1, G2 and G3. Also, the visual assay for PPO with catechol confirmed stability of *Ppo5* gene silencing from the initial generation through G2.

Thus, the stability of the inserts has been confirmed and trait stability monitoring will remain part of the commercial strategy for release of planting material for these events. Importantly, the stability of the inserts will not alter the pest characteristics of these events compared to conventional potato varieties and will not alter the environmental impact of potato production compared to conventional varieties.

REFERENCES

Sambrook J, Russell DW (2001) Southern Hybridization. In: Molecular Cloning: A Laboratory Manual. (Ed. Argentine J) Cold Springs Harbor Laboratory Press, Cold Springs Harbor, NY (pp. 6.33-6.38).

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.

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Appendix 4

Junction Analysis and Event-Specific Detection

INDEX

INTRODUCTION

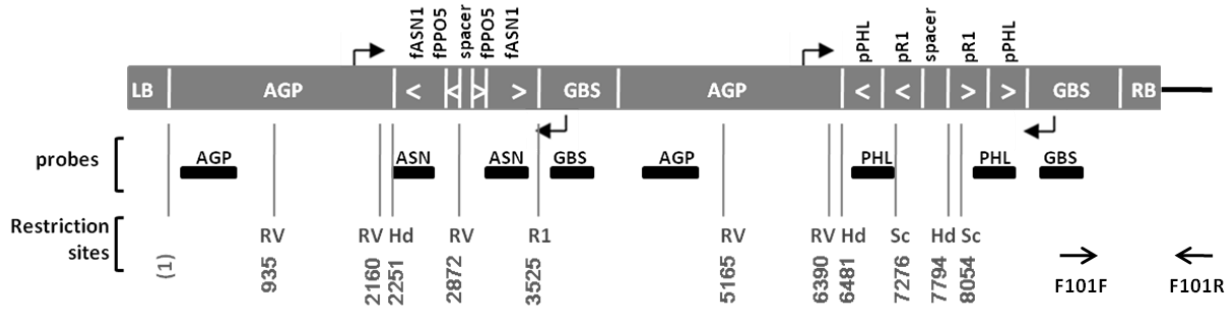
At least one DNA insert/flanking plant DNA junction was sequenced using either Adapter Ligation-Mediated PCR or Thermal Asymmetric Interlaced PCR. The junction sequence was used to design primers for each event, and these primers were applied for event-specific PCR-based detection methods. The specificity of the primers was tested on all the events submitted for deregulation to ensure that there was no cross-reactivity between the detection systems. The methods developed may be used to monitor plants and tubers in field and storage as part of quality management programs.

RESULTS

Ranger Russet Event F10

Based on the DNA insert organization of F10 (see **Figure 1** below and **Appendix 1. Characterization of Inserted DNA**), a fragment was amplified at the Right Border site. Sequence analysis determined that the DNA insert lacked the first 31 bp upstream from the Right Border cleavage site.

Figure 1. F10 Insert

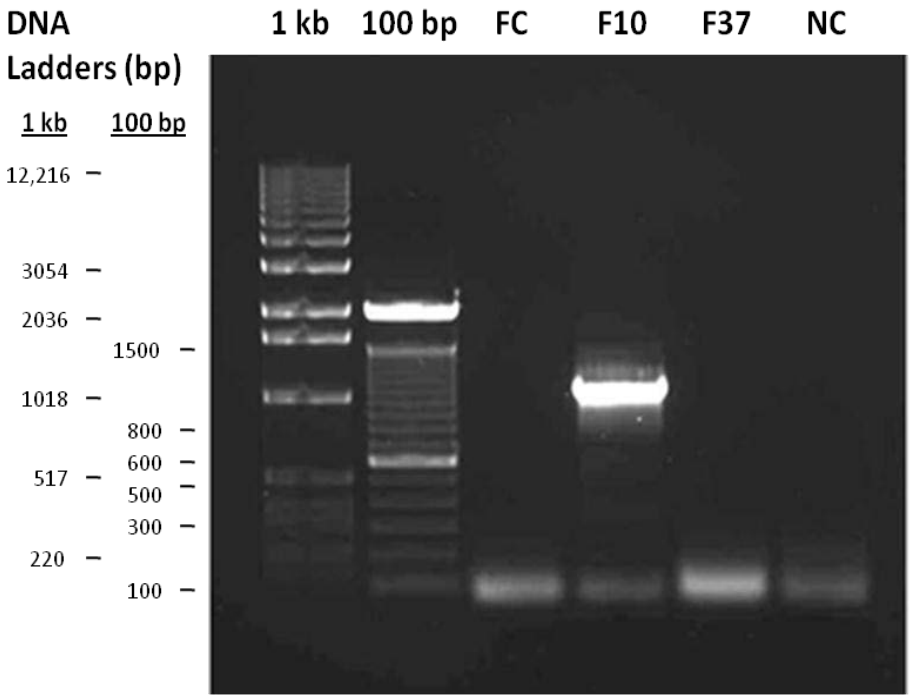


Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

The primers used for line-specific PCR were F10 1F, which is derived from the DNA insert, and F10 1R from the flanking plant DNA (**Figure 1**). The amplified DNA fragment for the line-specific PCR had a size of 1076 bp (**Figure 2**).

The single DNA insert of F10 had a 31 bp deletion at the Right Border side that did not affect the functional activity of the adjacent silencing cassette.

Figure 2. Ranger Russet F10: Line-Specific PCR Using Primer Pair F10 1F and F10 1R

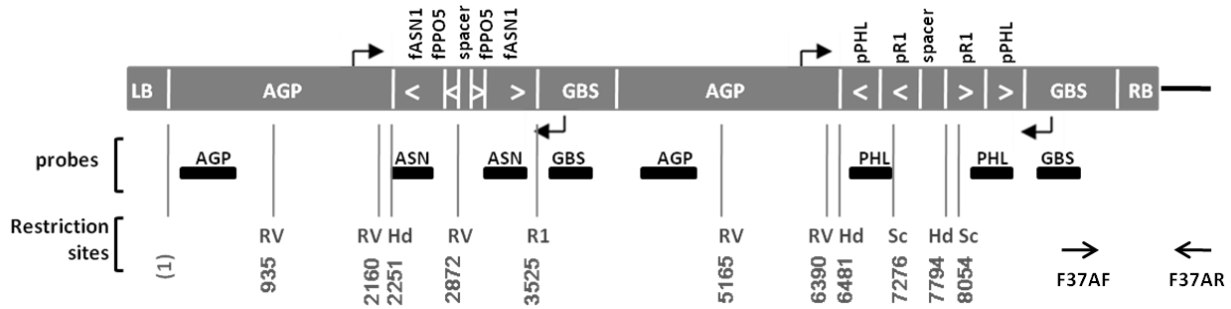


1kb = 1 kb DNA marker lane (Invitrogen), 100 bp = 100 bp DNA marker lane (invitrogen), FC = Ranger Russet control (untransformed), NC = negative control PCR reaction (without plant DNA).

Ranger Russet Event F37

Based on the DNA insert organization of F37 (see **Figure 3** below and **Appendix 1. Characterization of Inserted DNA**), a fragment was amplified at the Right Border site. The junction fragment consists of a truncated Right Border side of the DNA insert (which means that the integrated DNA insert lacks the Right Border and a small adjacent upstream sequence of 13 bp) and is followed by, first, a 13 bp plant-derived 'stuffer' DNA and, second, a small fragment (33 bp) of the DNA insert that represents an internal sequence of the trailer of the *Ppo* gene that is present in the silencing cassette of pSIM1278.

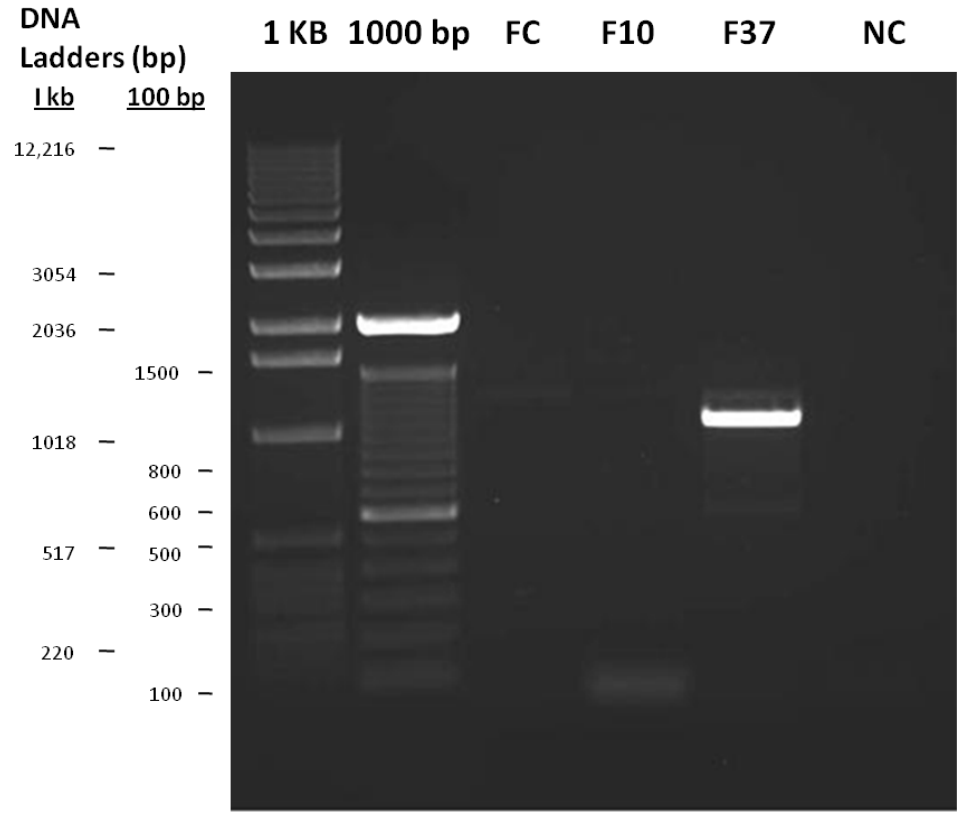
Figure 3. F37 Insert



Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

Two primers, one from the DNA insert and another from the sequenced flanking DNA, F37AF and F37AR (**Figure 3**), were used to amplify a 1091 bp F37-specific junction fragment (**Figure 4**).

Figure 4. Ranger Russet F37: Line-Specific PCR Using Primer Pair F37 AF and F37 AR. Amplified F37-specific fragment at Right Border side.

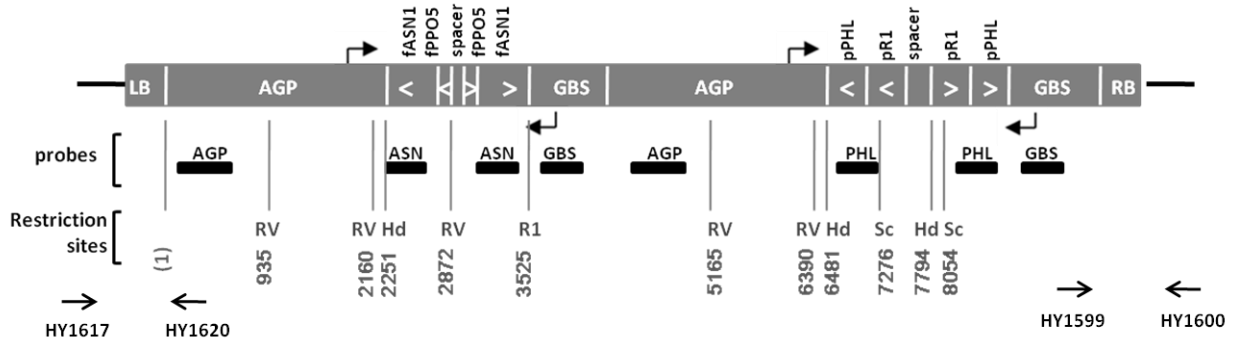


1kb = 1 kb DNA marker lane (Invitrogen), 100 bp = 100 bp DNA marker lane (invitrogen), FC = Ranger Russet control (untransformed), NC = negative control PCR reaction (without plant DNA).

Russet Burbank Event E12

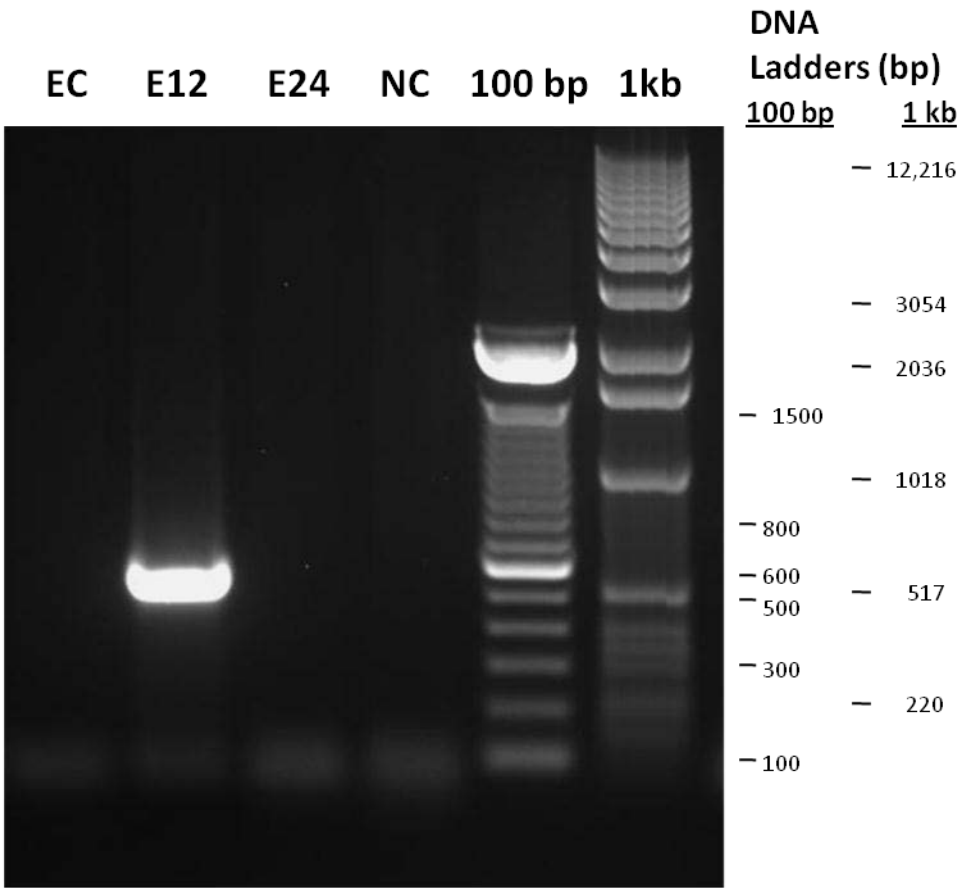
The DNA insert organization of E12 is shown in **Figure 5** below. A 547 bp fragment at the Right Border site lacked the first 92 bp upstream from the Right Border cleavage site. A 609 bp fragment at the Left Border site lacked 17 bp downstream from the Left Border cleavage site (**Figure 6A** and **B**).

Figure 5. E12 Insert



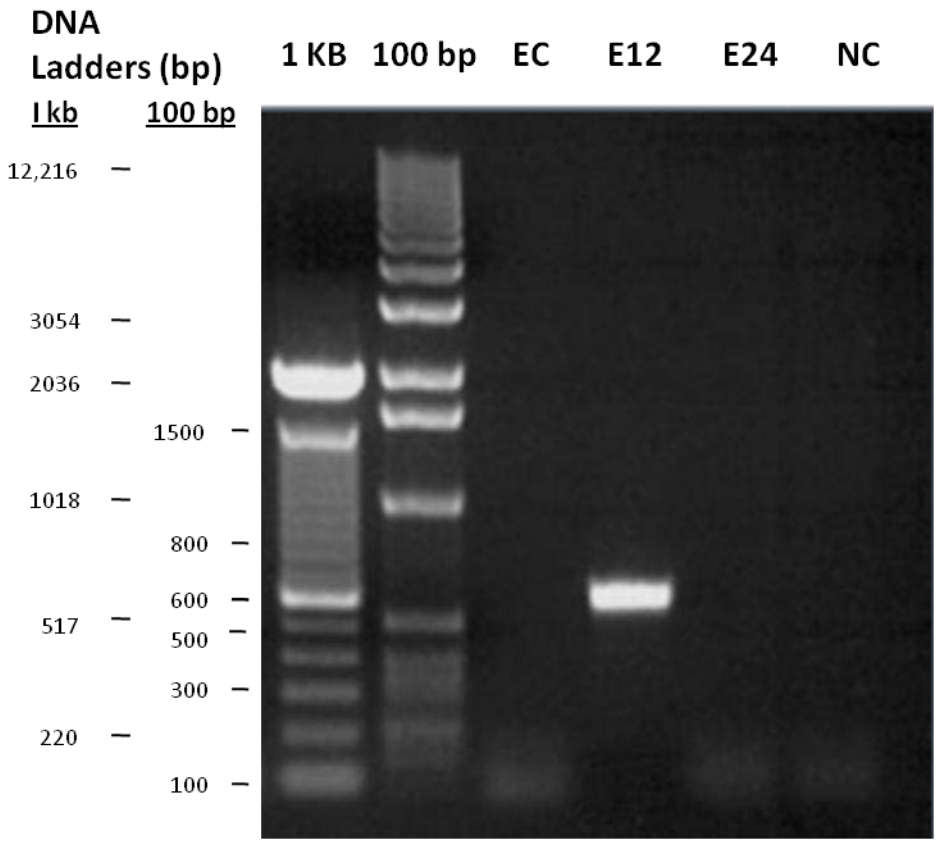
Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

Figure 6A. Russet Burbank E12: Line-Specific PCR. Amplified E12-specific fragment at the Right Border side, using primers HY1599 and HY1600.



1kb = 1 kb DNA marker lane (Invitrogen), 100 bp = 100 bp DNA marker lane (invitrogen), EC = Russet Burbank control (untransformed), NC = negative control PCR reaction (without plant DNA).

Figure 6B. Russet Burbank E12: Line-Specific PCR. Amplified E12-specific fragment at Left Border side using primer HY1617 and HY1620.

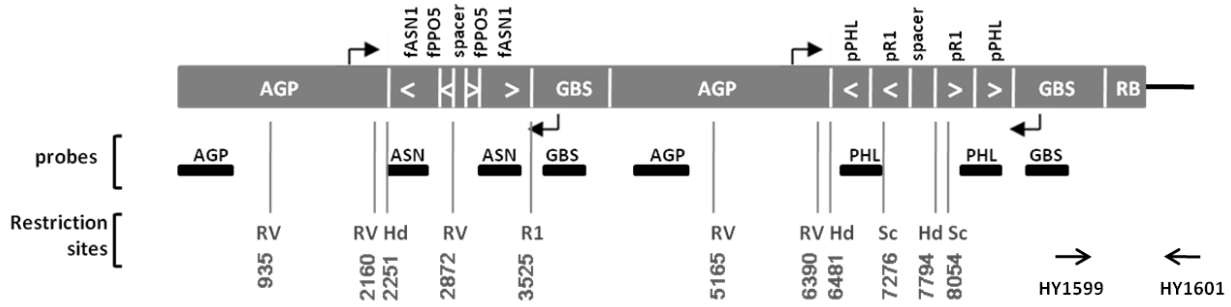


1kb = 1 kb DNA marker lane (Invitrogen), 100 bp = 100 bp DNA marker lane (invitrogen), EC = Russet Burbank control (untransformed), NC = negative control PCR reaction (without plant DNA).

Russet Burbank Event E24

TAIL-PCR was used to isolate DNA insert junction fragments from event E24 using the nested, Right Border side-specific primers HY1557, HY1558, HY1576 and HY1577 (**Table 1**). Based on the differential shift between the second and third products on agarose gels, E24-specific PCR products were extracted and cloned. Sequence analysis of the junction fragment demonstrates that E24 carries a full-length DNA insert (up to the cleavage site within the Right Border). Two primers, one annealing to a sequence within the DNA insert (HY1599) and one annealing to a site in the adjacent plant DNA (HY1601) (see **Appendix 1. Characterization of Inserted DNA**, **Figure 7** shown below), were used to amplify a 543 bp E24-specific DNA fragment **(Figure 8**).

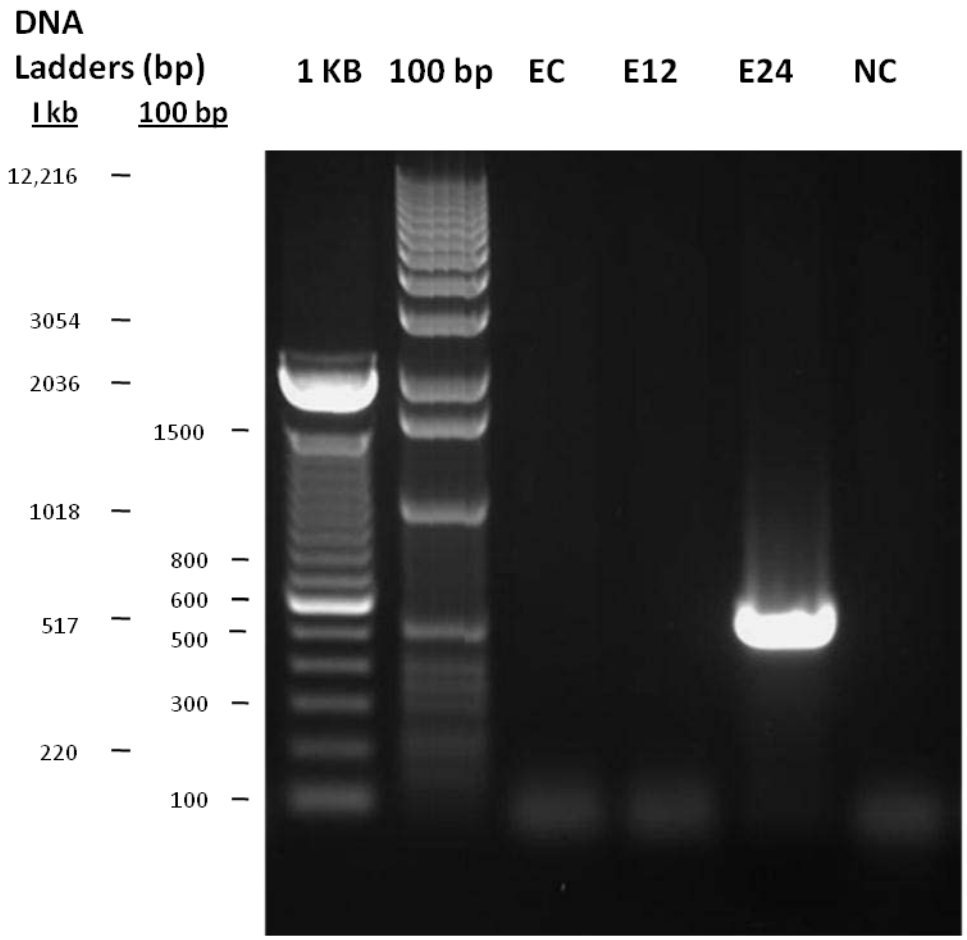
Figure 7. Insert for E24



Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

Sequence analysis demonstrated that the primers used for line-specific PCR are the DNA insert specific primer HY1599 and HY1601 which is from flanking DNA-derived DNA (**Figure 7**).

Figure 8. Russet Burbank E24: Line-Specific PCR Using Primer Pair HY1599 and HY1601. Amplified E24-specific fragment at Right Border side.

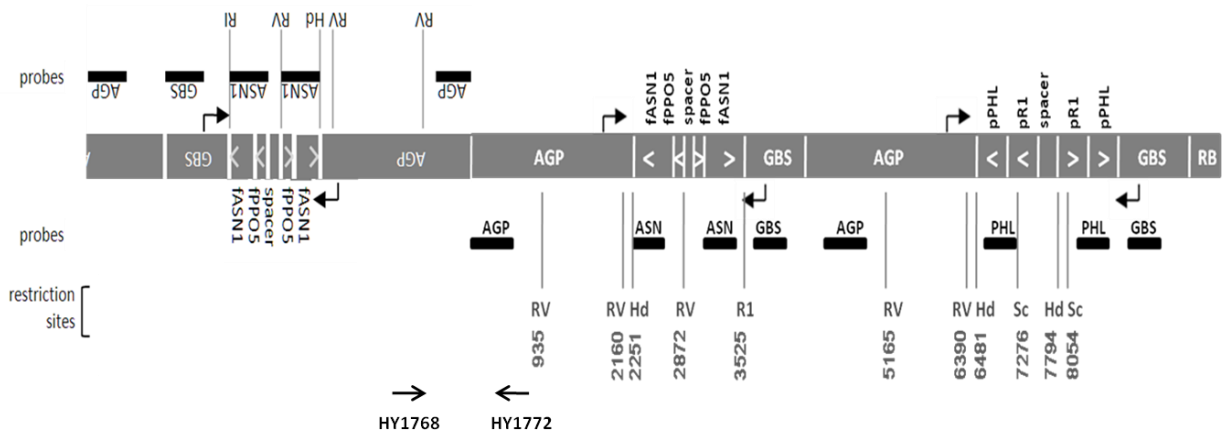


1kb = 1 kb DNA marker lane (Invitrogen), 100 bp = 100 bp DNA marker lane (invitrogen), EC = Russet Burbank control (untransformed), NC = negative control PCR reaction (without plant DNA).

Atlantic Event J3

Event J3 contains one nearly intact and one partial copy of the DNA insert, connected head to head at the Left Border site, with deletions of Left Border regions and parts of the adjacent Agp promoters (see **Appendix 1. Characterization of Inserted DNA** and **Figure 9** below).

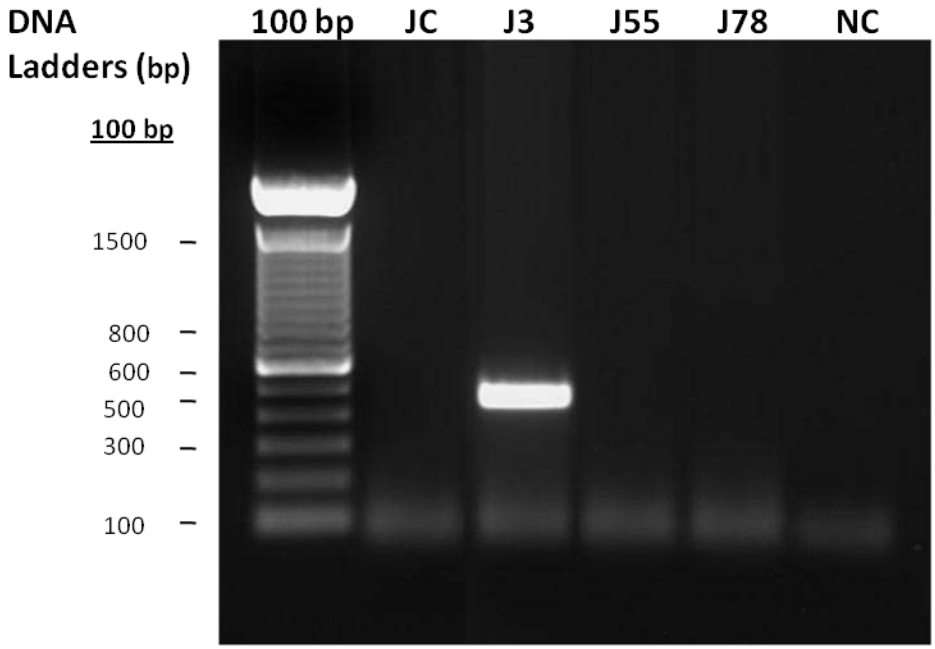
Figure 9. J3 Insert



Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

Different combinations of primers at the ends of the inserts were used to amplify DNA fragments, which were then analyzed by sequencing. It was found that one copy had a 430 bp deletion at the Left Border side while the other copy showed a 251 bp deletion at the Left Border side. The resulting structure comprises an inverted repeat carrying two truncated Agp promoters whereby the longer promoter fragment contains a 179 bp sequence that functions as spacer. To develop a line-specific PCR method, we designed one primer, HY1768, that anneals to an Agp promoter sequence on the one side of the connection and a second primer, HY1772, annealing to the Agp on the other side (**Figure 9**). As shown in **Figure 10,** these primers could be used to amplify a line specific 464 bp DNA fragment from J3 DNA but not from DNA of untransformed Atlantic, nor events J55 or J78.

Figure 10. Atlantic J3: Line-Specific PCR Using Primer Pair HY1768 and HY1772. Amplified J3-specific fragment between two DNA inserts at Left Border sides.

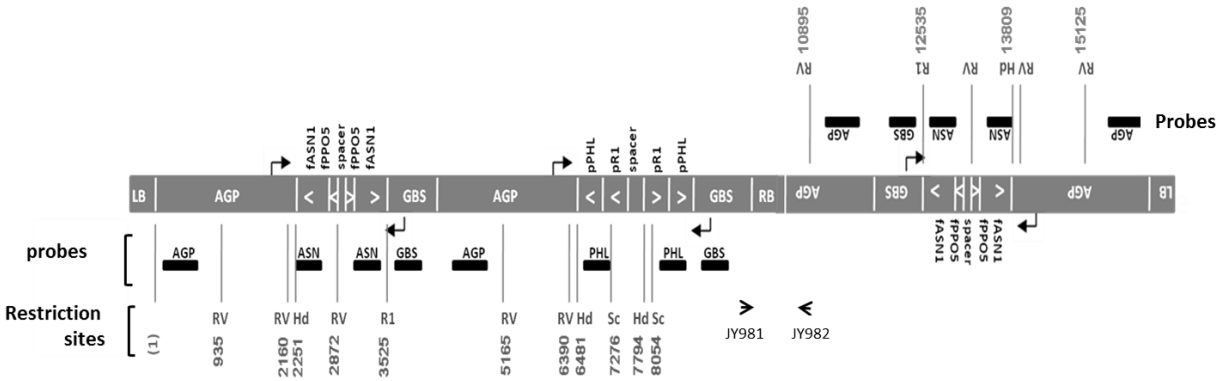


100 bp = 100 bp DNA marker lane (invitrogen), JC = Atlantic control (untransformed), NC = negative control PCR reaction (without plant DNA).

Atlantic Event J55

Both Southern analyses and TAIL PCR indicated that event J55 had two connected copies of the DNA insert. The Right Border region of the first DNA insert is connected to a truncated internal Agp region of the second DNA insert in the reverse orientation (see **Figure 11** below and **Appendix 1. Characterization of Inserted DNA**).

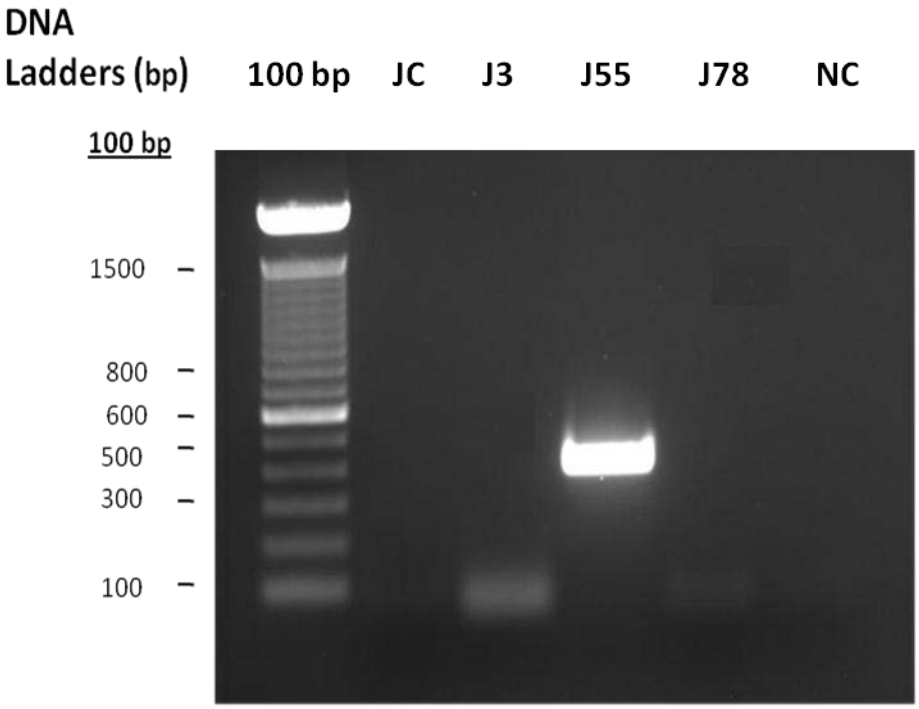
Figure 11. J55 Insert



Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

A short plant-derived 49 bp stuffer sequence is present between the two DNA inserts. The J55-specific forward primer JY981 was designed to anneal to the right side of the Gbss promoter of the first DNA insert and was 29-nt away from the left side of RB. The J55-specific reverse primer JY982 was targeted to the Agp promoter of the second DNA insert and was 187 nucleotides away from the right side of RB (**Figure 11**). As shown in **Figure 12**, a line specific 401 bp DNA fragment was amplified with these primers in event J55 but neither in untransformed Atlantic nor in J3 or J78.

Figure 12. Atlantic J55: Line-Specific PCR Using Primer Pair JY981 and JY982. Amplified J55-specific fragment.

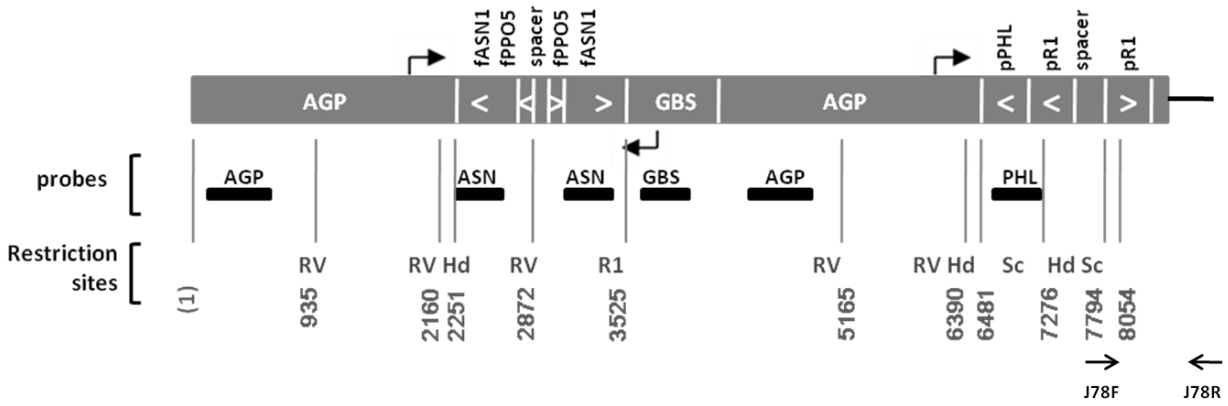


100 bp = 100 bp DNA marker lane (invitrogen), JC = Atlantic control (untransformed), NC = negative control PCR reaction (without plant DNA).

Atlantic Event J78

Based on the truncated structure of the DNA insert of J78 (see **Figure 13** below and **Appendix 1. Characterization of Inserted DNA**), a fragment of DNA flanking the Right Border side was sequenced. This sequence was used to design a primer, J78R, that, together with the DNA insert primer J78F (**Figure 13**) was used to amplify a J78-specific DNA fragment.

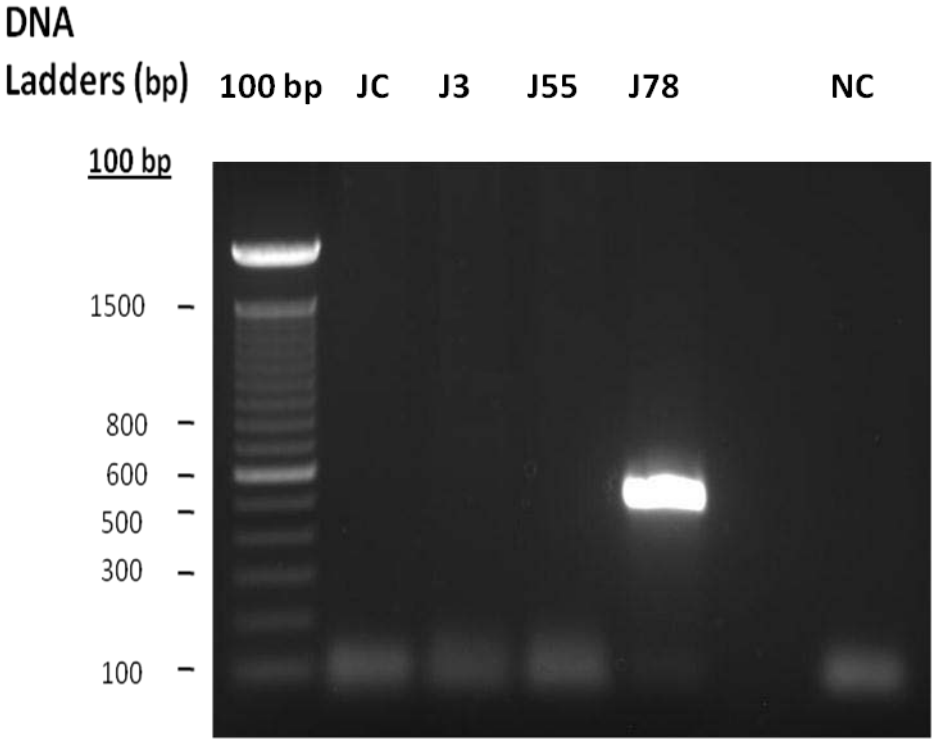
Figure 13. J78 Insert



Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

As shown in **Figure 14**, the 513 bp DNA fragment amplified with primers J78F and J78R was specific for J78. Sequencing the junction demonstrated that the DNA insert had a 1583 bp deletion at the Right Border side.

Figure 14. Atlantic J78: Line-Specific PCR Using Primer Pair J78F and J78R. Amplified J78-specific fragment at Right Border side.

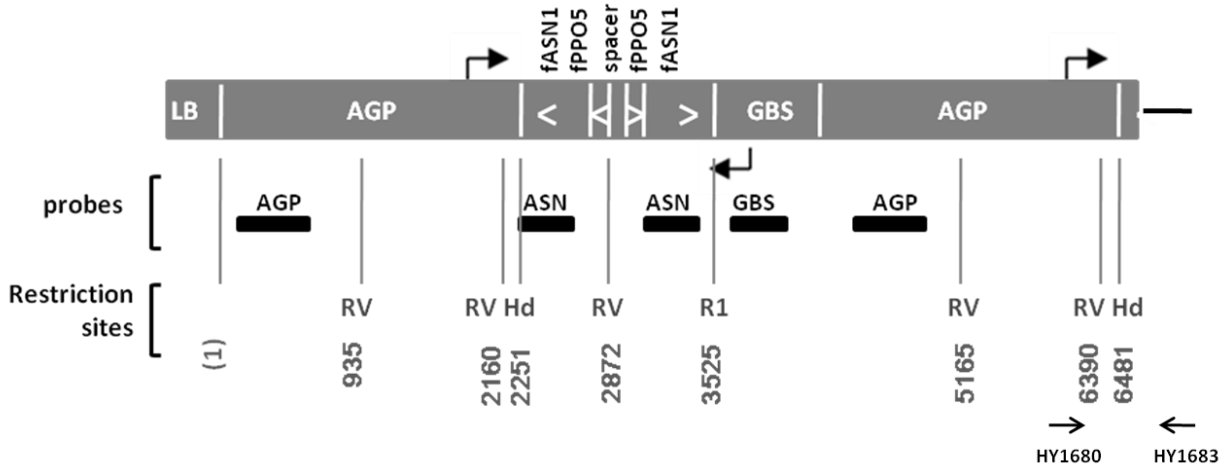


100 bp = 100 bp DNA marker lane (invitrogen), JC = Atlantic control (untransformed), NC = negative control PCR reaction (without plant DNA).

G event G11

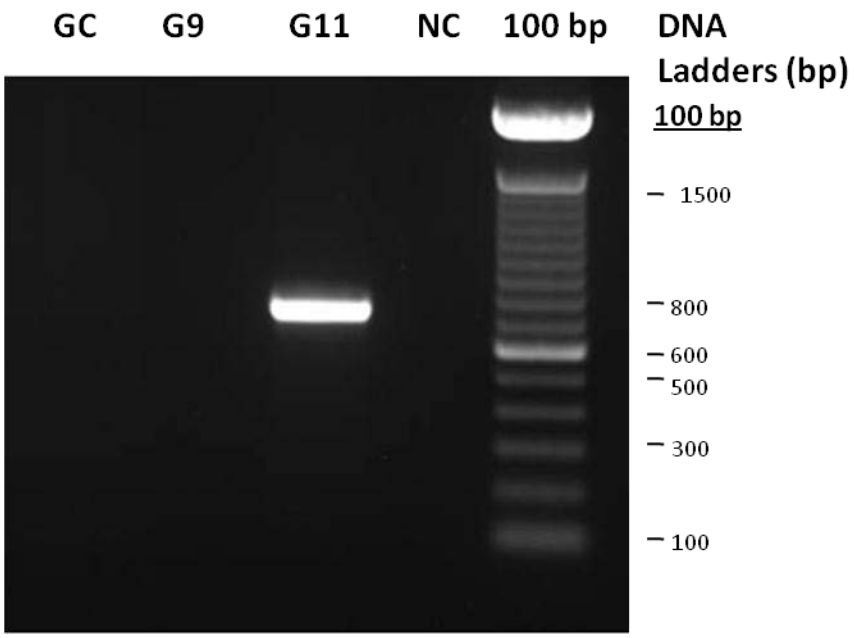
Based on the truncated structure of the DNA insert of G11 (see **Figure 15** below and **Appendix 1. Characterization of Inserted DNA**), the sequence of DNA flanking the Right Border side was determined. Two primers, one annealing to a sequence within the DNA insert (HY1680) and the other one annealing to flanking plant DNA (HY1683) (**Figure 15**), were used to amplify a 734 bp G11-specifc junction fragment (**Figure 16**). Sequence analysis of the fragment demonstrated that the DNA insert lacked 3,317 bp at the Right Border side, which means that it contained a full *Asn1*/*Ppo5* gene silencing cassette and lacked the R1/PhL promoter silencing cassette.

Figure 15. Insert for G11



Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

Figure 16. G11: Line-Specific PCR Using Primer Pair HY1680 and HY1683. Amplified G11-specific fragment (734 bp) at Right Border side.

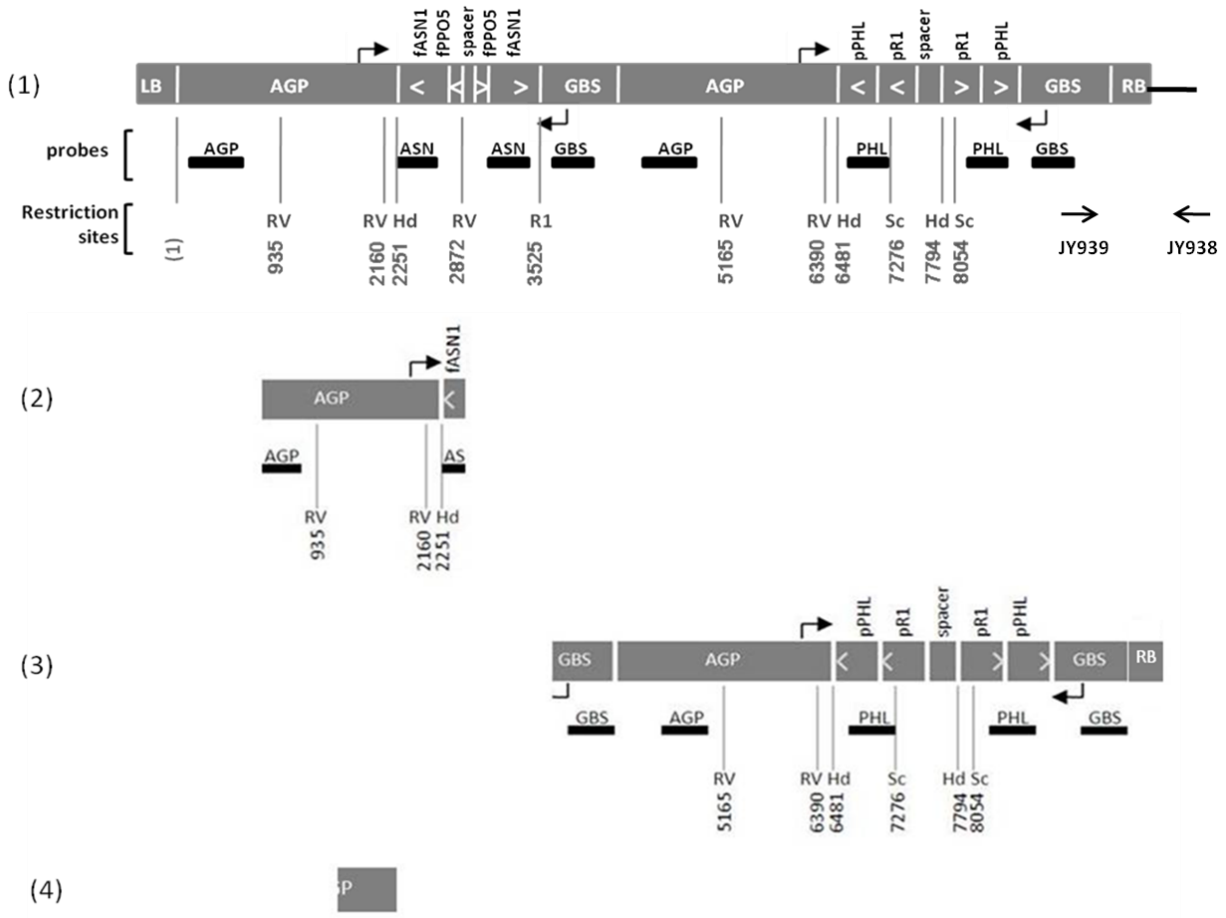


100 bp = 100 bp DNA marker lane (invitrogen), GC = G control variety (untransformed), G9 = G event, not included in this submission, NC = negative control PCR reaction (without plant DNA).

H event H37

Based on the DNA insert organization of H37 (see **Figure 17** below and **Appendix 1. Characterization of Inserted DNA**), a Right Border junction fragment was isolated using the nested primers (HY1557, HY1558, and HY1576) by TAIL-PCR. Sequencing of the fragment made it possible to design two primers, one annealing to a sequence within the DNA insert (JY939) and the other one annealing to a flanking plant DNA sequence (JY938) (**Figure 17**).

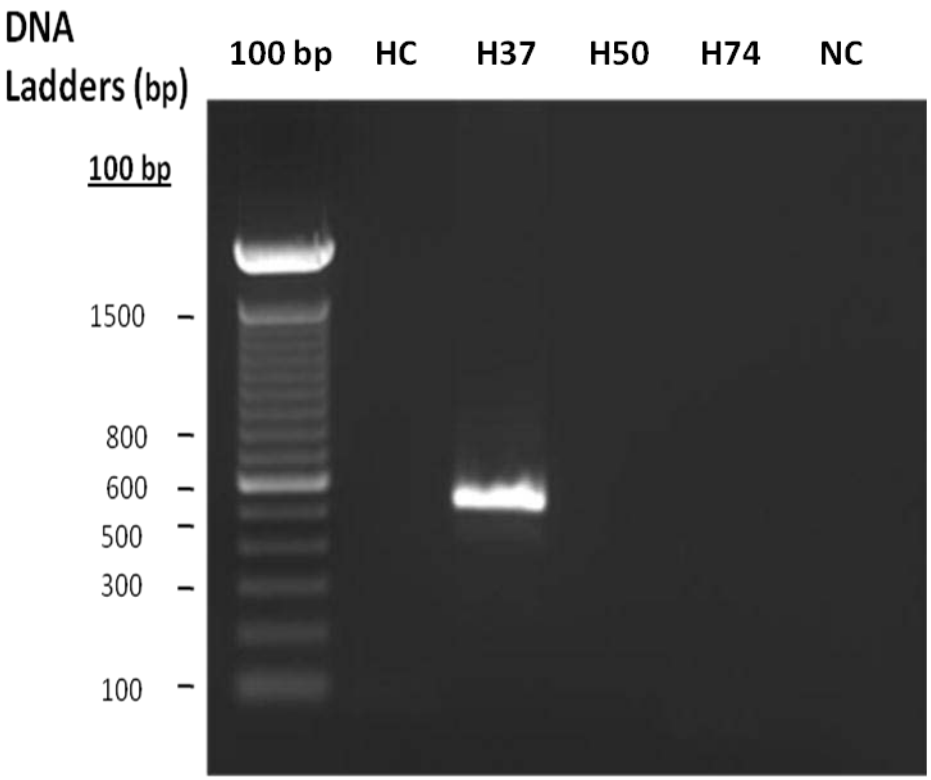
Figure 17. H37 Insert



Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

The event-specific primers were used to amplify the junction. As demonstrated in **Figure 18**, the 553 bp DNA fragment was amplified with H37 DNA but not with DNA from either the conventional variety H or the event H50.

Figure 18. H37: Line-Specific PCR Using Primer Pair JY938 and JY939. Amplified H37-specific fragment (553 bp) at Right Border side.

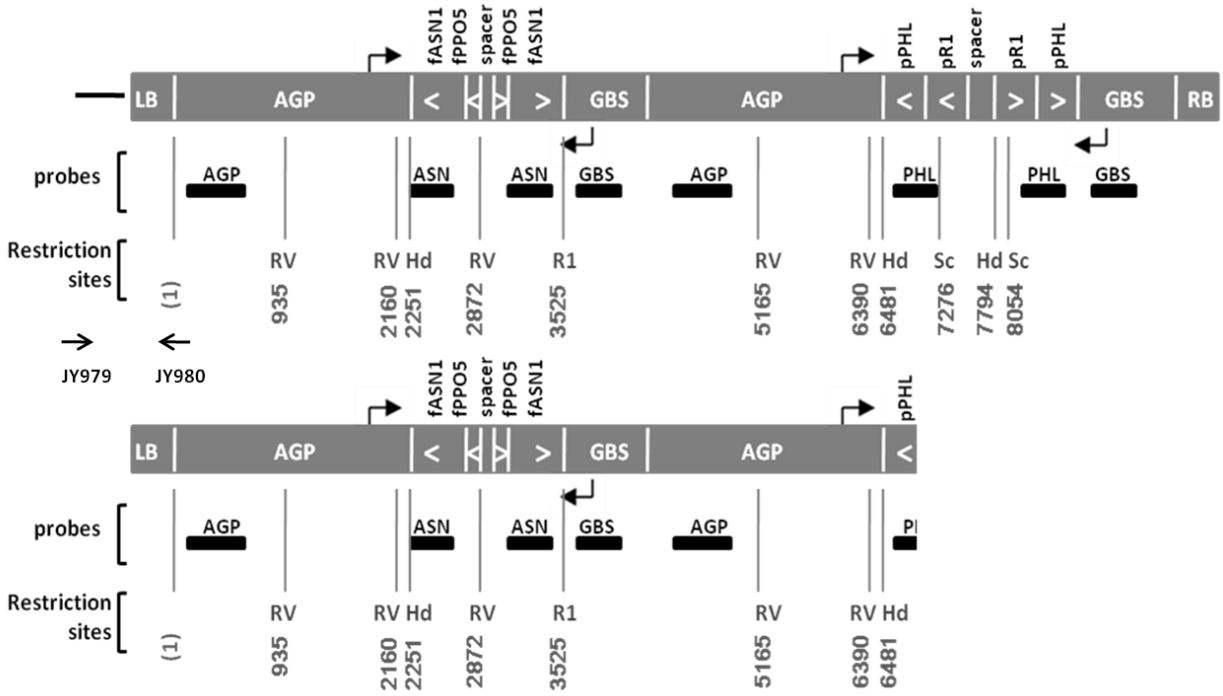


100 bp = 100 bp ladder (Invitrogen), HC = H Control, H74 = H event not included in this submission, NC = negative control (PCR reaction without plant DNA).

H event H50

The DNA insert organization of H50 is shown in **Figure 19** below and **Appendix 1. Characterization of Inserted DNA**. A junction fragment was isolated using TAIL-PCR with the nested primers HY1562, HY1566, and HY1567 (**Table 2**).

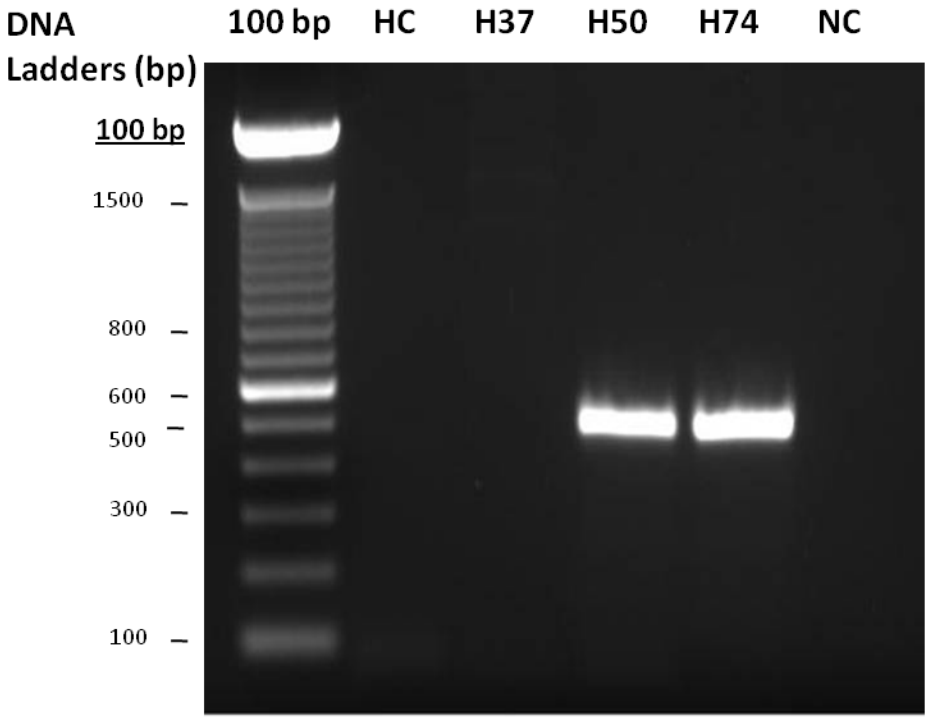
Figure 19. Insert for H50



Arrows below the diagram (→) indicate the approximate position of primers used to amplify an event-specific DNA fragment. Promoter orientation indicated by arrows (⟶).

Based on the size difference between the first and second PCR products on agarose gels, specific PCR products were cloned and sequenced. The H50 Left Border junction specific primers JY979 and JY980 were used to amplify a line specific 507-bp junction fragment in the H50 event but not in the conventional variety H nor H37 (**Figure 20)**.

Figure 20. H50: Line-Specific PCR Using Primer Pair JY979 and JY980. Amplified H50-specific fragment at Left Border side.

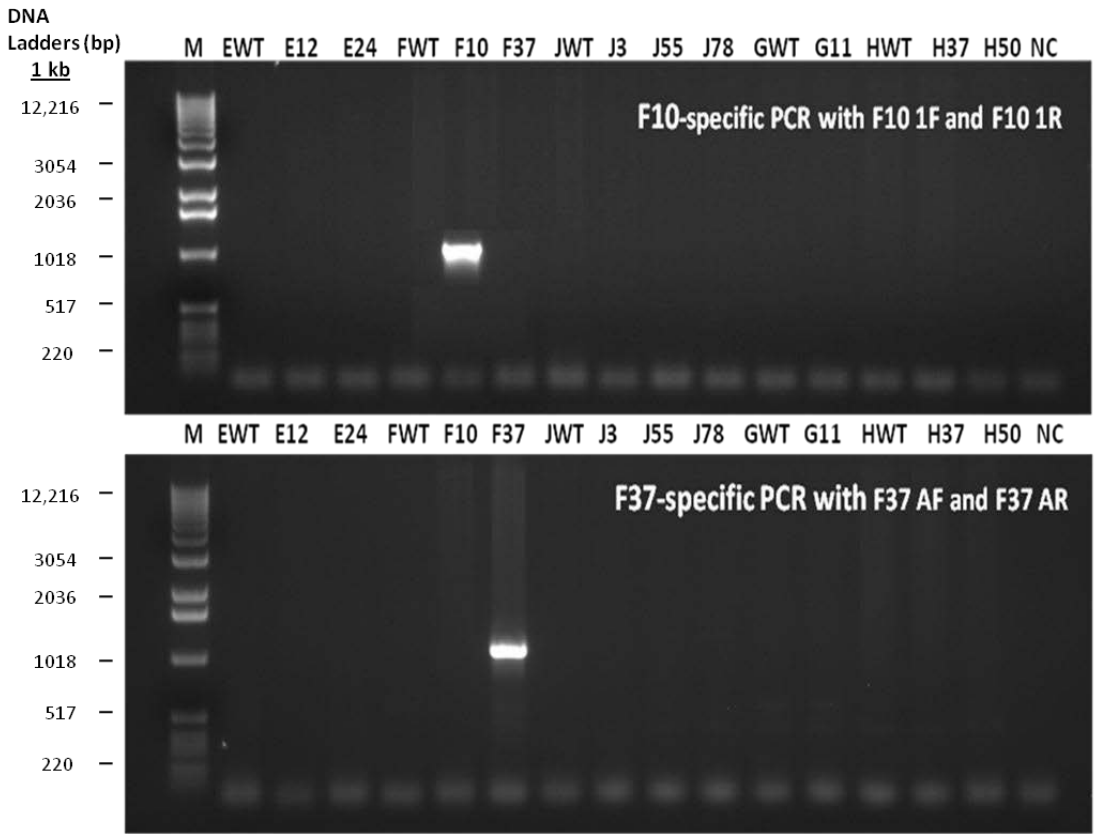


M = 100 bp ladder (Invitrogen), HC = H Control, H74 = event not included in this submission, NC = negative control (PCR reaction without plant DNA).

Line-Specificity of PCR-Based Detection Methods.

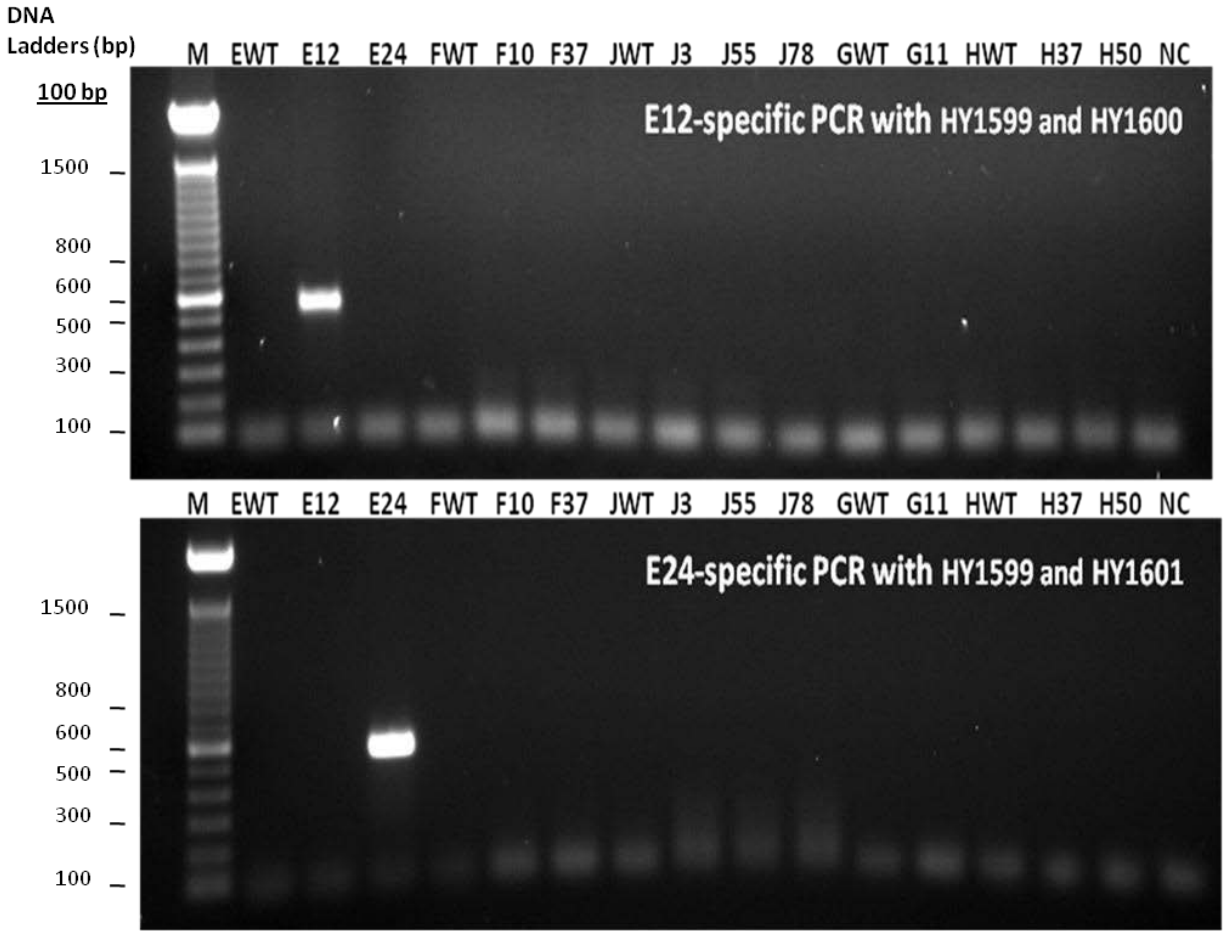
As shown in **Figure 21**, the 10 event-specific PCR methods do not cross react with the other events in this deregulation submission.

Figure 21. Line-Specific PCR Detection Methods



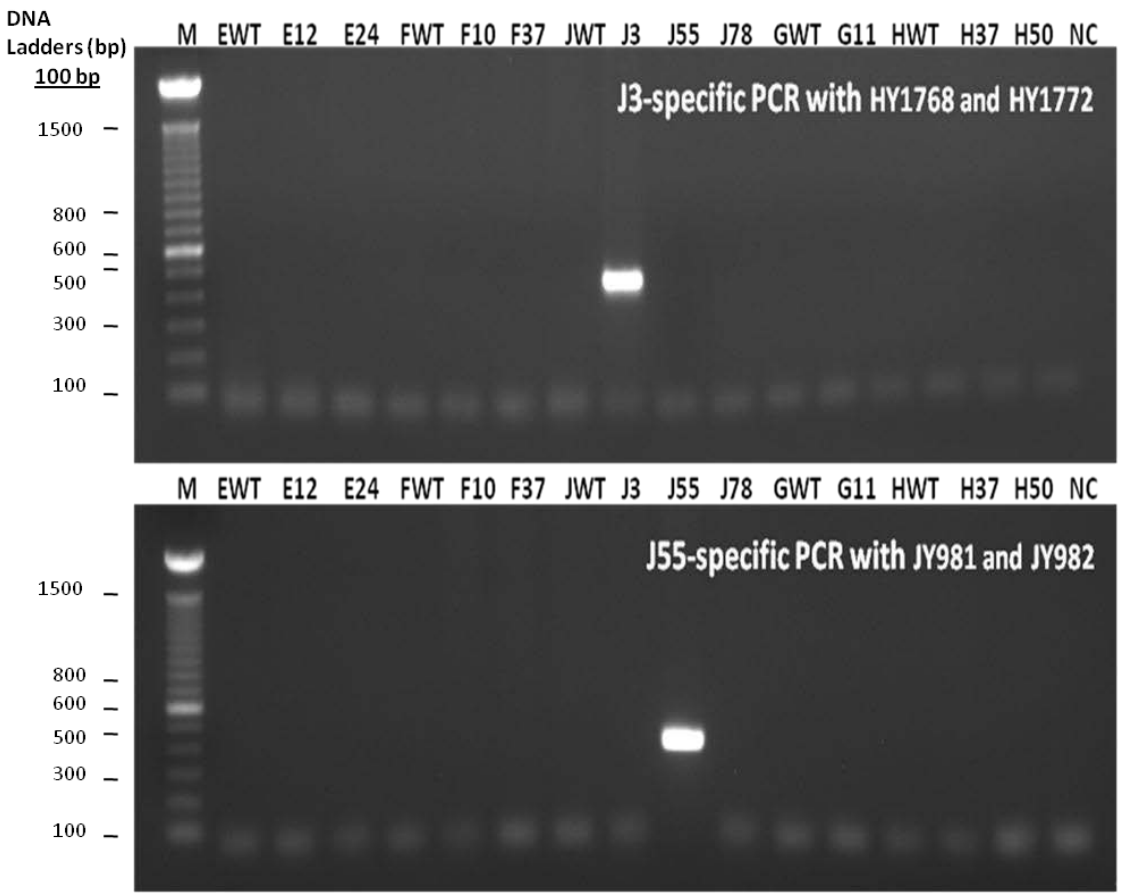
M = 100-bp ladder from invitrogen; EWT = Russet Burbank Control; FWT = Ranger Russet Control; JWT = Atlantic Control; GWT = G Control; HWT = H Control. NC = negative control (PCR reaction without plant DNA). Test lines as indicated in the text (F10, F37).

Figure 21 (Continued). Line-Specific PCR Detection Methods



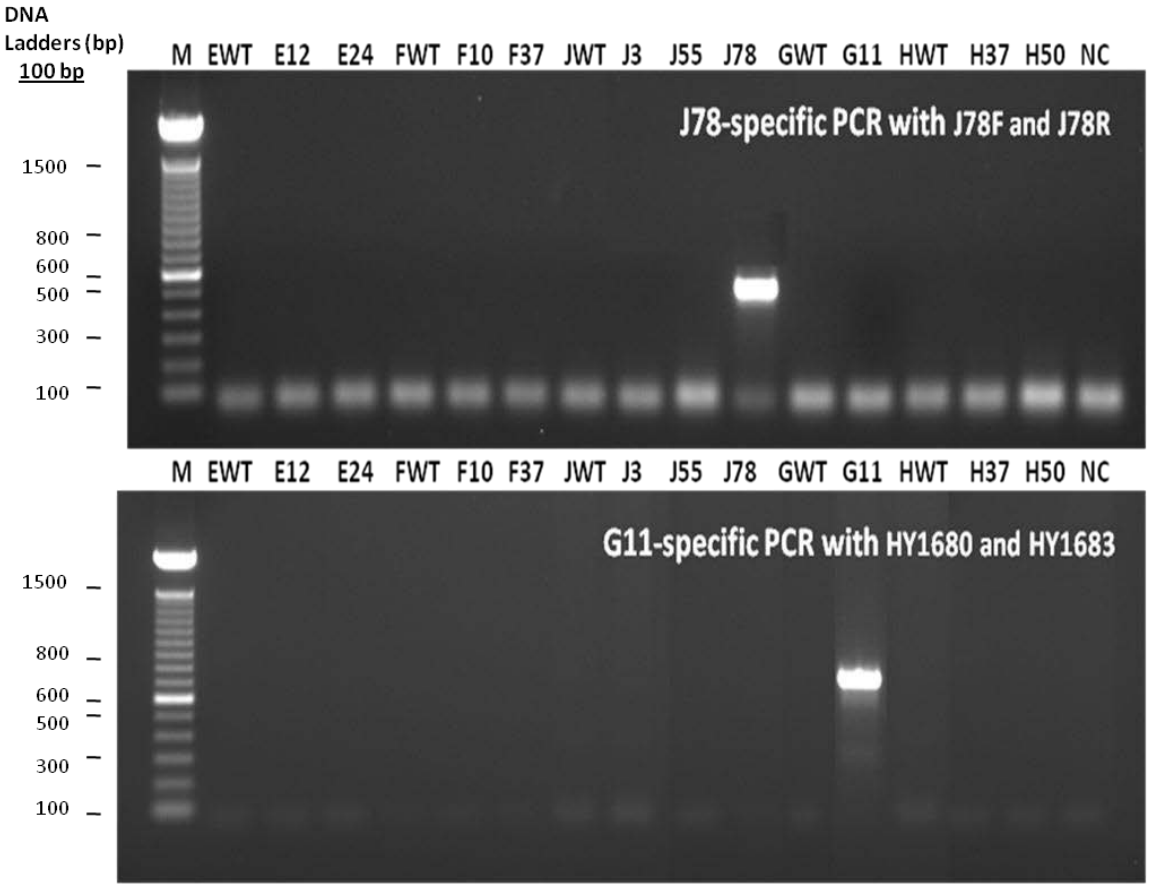
M = 100 bp ladder from invitrogen; EWT = Russet Burbank Control; FWT = Ranger Russet Control; JWT = Atlantic Control; GWT = G Control; HWT = H Control. NC = negative control (PCR reaction without plant DNA). Test lines as indicated in the text (E12, E24).

Figure 21 (Continued). Line-Specific PCR Detection Methods



M = 100 bp ladder from invitrogen; EWT = Russet Burbank Control; FWT = Ranger Russet Control; JWT = Atlantic Control; GWT = G Control; HWT = H Control. NC = negative control (PCR reaction without plant DNA). Test lines as indicated in the text (J3, J55).

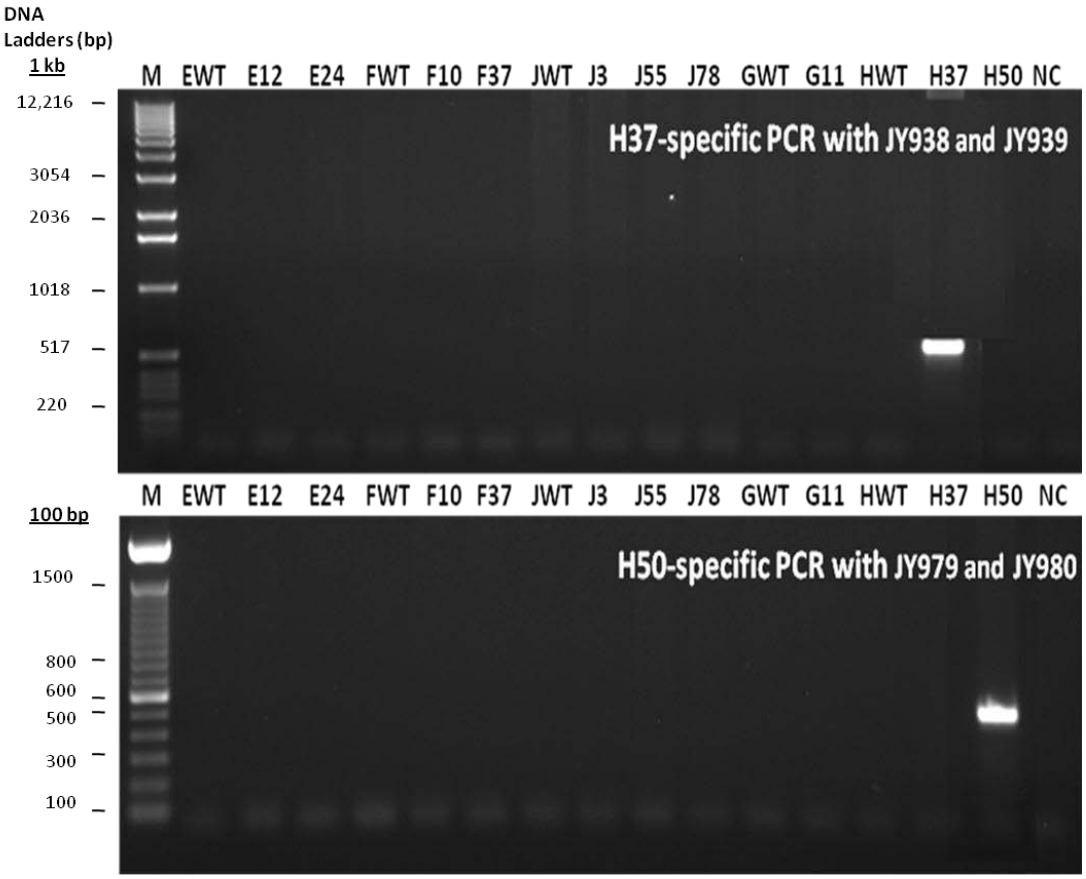
Figure 21 (Continued). Line-Specific PCR Detection Methods



M = 100 bp ladder from invitrogen; EWT = Russet Burbank Control; FWT = Ranger Russet Control; JWT = Atlantic Control; GWT = G Control; HWT = H Control. NC = negative control (PCR reaction without plant DNA). Test lines as indicated in the text (J78, G11).

Figure 21 (Continued). Line-Specific PCR Detection Methods

M =



100bp ladder from invitrogen; EWT = Russet Burbank Control; FWT = Ranger Russet Control; JWT = Atlantic Control; GWT = G Control; HWT = H Control. NC = negative control (PCR reaction without plant DNA). Test lines as indicated in the text (H37, H50).

MATERIALS AND METHODS

Potato material. Leaves from greenhouse-grown events and untransformed plants were used to extract DNA and isolate and characterize the DNA insert junctions.

Adapter ligation-mediated PCR Junction fragments were amplified by PCR using digested DNA ligated with adapter primers AP1 and AP2 as described by O'Malley *et al*. (2007) and detailed below. The amplified product containing a DNA insert primer binding site was selected by a secondary PCR reaction. The sequences of DNA insert primers used to analyze RB and LB junctions are shown in **Tables 1** and **2**, respectively. Plant DNA was isolated using either the CTAB method or the Qiagen Plant DNAeasy kit: 200-500 ng DNA was digested for 3-5 hours with a restriction enzyme for which an adapter had been designed (*Eco*RI, *Hin*dIII, *Bam*HI, *Ase*I/*Nde*I) in a total volume of 20 µl. The digested DNA (3 µl) was ligated with its respective adapter (1 µl) in a mixture containing 0.5 µl T4 DNA ligase, 0.3 µl ATP, 1.0 µl 10X ligation buffer, and 4.2 µl water. The primary PCR was carried out using the first DNA insert specific primer and AP1 with Hot Master Taq polymerase (Fisher BioReagents) under the following amplification conditions: 1 cycle of 3 min at 95°C followed by 30 cycles of 30 sec at 94°C, 30 sec at 60°C, 2.5 min at 68°C, and finishing with 10 min at 68°C. A 1 µl aliquot of the primary PCR product was used for secondary reactions (1 cycle of 3 min at 95°C; 35 cycles of 30 sec at 94°C, 30 sec at 62°C, 2.5 min at 68°C; 1 cycle of 10 min at 68°C) with the second DNA insert specific primer and AP2. This protocol was performed at the same time for non-transformed control plants and the events. Products of the secondary PCR were run on 1% agarose in TAE buffer. Bands unique for the events were gel-extracted using a Qiagen QIAquick Gel Extraction kit, cloned into pGEM-T Easy vector (Promega, Madison WI), and sequenced. Primers specific for DNA insert-flanking DNA were used, together with a DNA insert primer, to amplify junction fragments.

Table 1. DNA Insert Primers Used to Analyze RB Junction Fragments

Primer Name	Primer position in pSIM1278
PHL-GBSS	8822- 8844
GBSS-PR	9756- 9777
HY1669	1675 - 1698 ; 5905- 5928
HY1670	6479- 6504
HY1671	6516- 6541
HY1557	8824- 8848
HY1558	4011- 4035; 9320- 9344
HY1576	9751- 9777
HY1577	9846- 9869
RB3R-T1a	7696- 7719
RB3L-T1b	7736- 7759
RB3R-T1d	7866- 7889

Table 2. DNA Insert Primers Used to Analyze LB Junction Fragments

Primer Name	Primer position in pSIM1278
HY1562	460- 485; 4690- 4715 (reverse)
HY1566	19643-7 (reverse)
HY1567	19590- 19614 (reverse)

Thermal asymmetric interlaced (TAIL) PCR. This method was used as an alternative to adapter ligation-mediated PCR. Three series of reactions using AD (Arbitrary Degenerate) and SP (nested sequence-specific) primers were carried out to obtain DNA insert junction fragments. The standard 20 µl reaction mixture consisted of 2 µl 10X PCR buffer, 0.4 µl 10 mM dNTP, 0.4 µl 10 µM SP1, 5 µl 8-16 µM AD, 1 µl template, with either 0.1 µl HotMaster Taq polymerase (extension temperature 68 °C) or *TaKaRa Ex* Taq™ (TAKARA BIO Inc). A 1 µl aliquot of 50ng/µl leaf DNA was used as template for the first PCR; 1 µl of a 1:50 dilution of the first reaction was used as template for the second PCR; and 1 µl of 1:50 dilution of the second reaction was used for the third PCR. The second and third reactions were run on a gel, and bands with sizes that changed between the second and third reactions were cloned into pGEM-T Easy vector (Promega, Madison WI) and sequenced. Primers specific for DNA insert-flanking DNA were used, together with DNA insert-specific primers to clone junction fragments that were sequenced to confirm their identity.

CONCLUSIONS

At least one junction where the DNA insert meets the flanking plant DNA was sequenced for each of the 10 events and those sequences were used to design event-specific PCR-based detection methods (see summary of primers for each event in **Table 3**). The specificity of the primers was tested on all the events to ensure that there was no cross-reactivity with other events. The methods may be used to monitor plants and tubers in field and storage as part of quality management programs and can be developed further to provide for routine testing in food.

Table 3. Primers Used for Event-specific PCR

Event	Forward Primer	Reverse Primer	PCR Product (bp)
F10	F101F	F101R	1076
F37	F37AF	F37AR	1091
E12 (Left Border)	HY1617	HY1620	609
E12 (Right Border)	HY1599	HY1600	547
E24	HY1599	HY1601	543
J3	HY1768	HY1772	464
J55	JY981	JY982	401
J78	J78F	J78R	513
G11	HY1680	HY1683	734
H37	JY939	JY938	553
H50	JY979	JY980	507

Event Ranger Russet F10: Primers F101F and F101R can be used to amplify an F10-specific DNA fragment of 1076 bp, and thus results in a line specific test method for event F10.

Event Ranger Russet F37: Primers F37AF and F37AR can be used to amplify an F37-specific DNA fragment of 1091 bp, and thus results in a line specific test method for event F37.

Event Russet Burbank E12: Primers HY1617 and HY1620 can be used to amplify an E12-specific DNA fragment of 609-bp at the Left Border, and primers HY1599 and HY1600 can be used to amplify a 547 bp fragment at the Right Border, and thus either set of primers could result in a line specific test method for event E12.

Event Russet Burbank E24: Primers HY1599 and HY1601 can be used to amplify an E24-specific DNA fragment of 543 bp, and thus results in a line specific test method for event E24.

Event Atlantic J3: Primers HY1768 and HY1772 can be used to amplify a J3-specific DNA fragment of 464 bp, and thus results in a line specific test method for event J3.

Event Atlantic J55: Primers JY981 and JY982 can be used to amplify a J55-specific DNA fragment of 401 bp, and thus results in a line specific test method for event J55.

Event Atlantic J78: Primers J78F and J78R can be used to amplify a J78-specific DNA fragment of 513 bp, and thus results in a line specific test method for event J78.

Event G11: Primers HY1680 and HY1683 can be used to amplify a G11-specific DNA fragment of 734 bp, and thus provide an event-specific test method for G11.

Event H37: Primers JY938 and JY939 can be used to amplify an H37-specific DNA fragment of 553 bp, and thus provide an event-specific test method for event H37.

Event H50: Primers JY979 and JY980 can be used to amplify an H50-specific DNA fragment of 507 bp, and thus provide an event-specific test method for event H50.

REFERENCES

O'Malley RC, Alonso JM, Kim CJ, Leisse TJ, Ecker JR (2007) An adapter ligation-mediated PCR method for high-throughput mapping of T-DNA inserts in the *Arabidopsis* genome. Nature Protocols, 2:2910-2917.

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.

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Appendix 5

Efficacy and Tissue-Specificity of Gene Silencing

INDEX

INTRODUCTION

Expression of the four genes targeted for gene silencing by transformation with pSIM1278 was tested in tubers, stolons, leaves, stems, roots, and flowers of the potatoes. The purpose of these studies was to show the effectiveness of gene silencing based on RNA expression as measured through Northern blot analyses. It's important to note that interpretation of the Northern blots requires judgment and the reported results represent a qualitative approach whereby the genes were considered downregulated. All down-regulated genes indicated in the summary (**Table 1)** appeared reduced when compared with the respective controls, without differentiation between reduction levels, as appropriate for a qualitative method. When both greenhouse and field tubers were analyzed, a judgment was made after reviewing both gels.

Considering the potato events in this study, expression of the targeted genes would provide evidence that the desired phenotype with low asparagine, reduced black spot bruise, and lowered reducing sugars had been achieved. It could provide information relevant to the plant pest risk assessment, for example, all Innate™ potatoes in the study had reduced or similar expression to the controls. Thus, the altered expression is unlikely to affect weediness or other plant pest characteristics. Also, with these events, the expression studies pertain to endogenous proteins rather than new proteins that could require a safety evaluation, such as those associated with herbicide tolerance or insect resistance. Gene silencing methods were employed to lower the activity of the Asn1, Ppo5, PhL, and R1 native proteins, and transcript levels rather than protein amounts were evaluated to link new phenotypic traits to changes at the molecular level. For this purpose, RNA was isolated from various tissues, and the RNA was used to carry out Northern blot analyses.

The promoters used to drive expression of the two inverted repeats, the Agp promoter and the Gbss promoter, have been well characterized and are known to be highly active in tubers and stolons while inducing some transcription in photosynthetically-active tissues and roots (Nakata *et al*. 1994; Visser *et al*. 1991). Therefore, silencing effects mediated by expression of the inverted repeats were predicted to be strong in tubers and stolons, and less pronounced in leaves, stems, roots, and flowers. The silencing effects in tissues other than tubers and stolons may have been further accentuated by the anticipated mobility of small RNAs produced by the silencing cassettes (Molnar *et al*. 2010).

Northern blot analyses were carried out to determine the actual effects of the transformations on expression levels of the four targeted genes in a tissue-specific manner for each event.

Most studies were carried out on tissues from greenhouse-grown material including tubers, stolons, roots, stems, leaves, and flowers. Additionally, some field-grown tubers were analyzed as well because tubers are the target tissues for down-regulation of the four genes.

RESULTS

Transcript Levels in Tubers

The transcript levels of target genes in various tissues of untransformed plants and the events were determined by Northern blot analysis.

In tubers of untransformed controls as viewed by Northern blot analyses, transcript levels were higher compared with the events for the *Asn1*, *Ppo5, PhL*, and *R1* genes. A comparison of northern blots indicated that the *Asn1*, *Ppo5*, and *PhL* were expressed at similar levels in tubers from greenhouse (**Figures 1A, 2A, 3A** and **4A**) and field (**Figures 1B, 2B, 3B** and **4B**). One exception was observed with *Ppo5* silencing, in which greenhouse-grown tubers had higher expression levels in both controls and events than in tubers from the field (**Figure 2B**). In contrast, the *R1* gene was only partially silenced, with slight differences observed between events and controls in either greenhouse-grown or tubers from the field.

Strongly reduced transcript levels for the *Asn1* and *Ppo5* genes in tubers of all events were intentional and are associated with low-acrylamide potential and black spot bruise tolerance (**Figures 1** and **2**). However, *Ppo5* gene transcripts in event H50 were slightly higher in greenhouse samples than in tubers from the field. This finding may be due to the fact that the H50 tubers from the greenhouse were not fully mature; some lingering activity of *Ppo* genes other than *Ppo5*, which are only expressed in immature tubers, may have caused residual transcript levels.

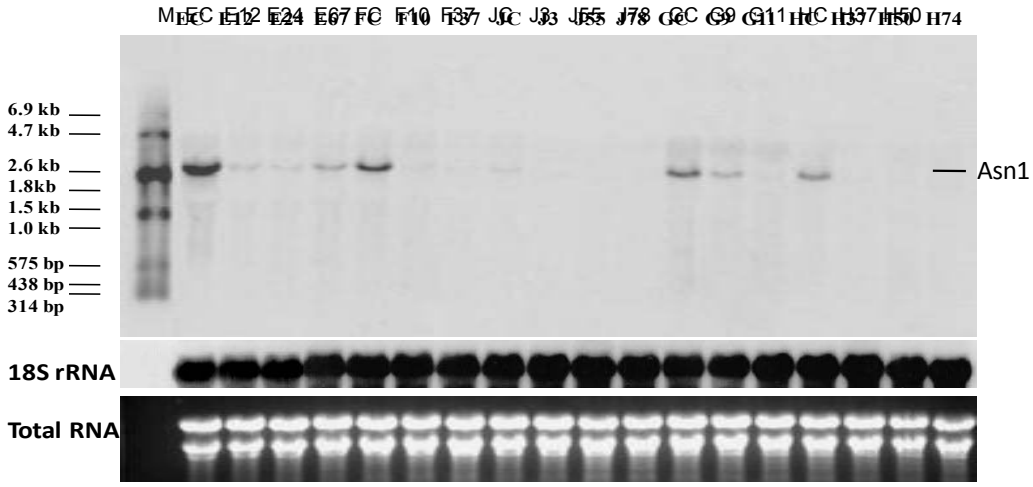
Transcript levels for the *PhL* gene were partially reduced in the tubers of most events (**Figure 3**), except F10, F37, J78, and G11. This reduced *PhL* was intentional, and is linked to reduced amounts of glucose and fructose. It was expected that J78 and G11 would not have reduced transcripts of *PhL*, yet F10 and F37 were expected to show silencing of that gene. *R1* transcripts were partially reduced ("whispered") in tubers of most lines to help limit the degradation of starch into sugars (**Figure 4**). Results varied between greenhouse and field grown tubers, yet the overall conclusion was that some silencing of *R1* occurred in most events.

Events J78 and G11 lack a functional PhL/R1 promoter silencing cassette and, consequently, display no reductions in transcript levels for the *PhL* and *R1* genes (**Figures 3** and **4**). The lack of silencing of *PhL* in F10 and F37, along with slight reductions of R1 in most events, show that generally promoter silencing of these genes did not down-regulate genes as much as expected.

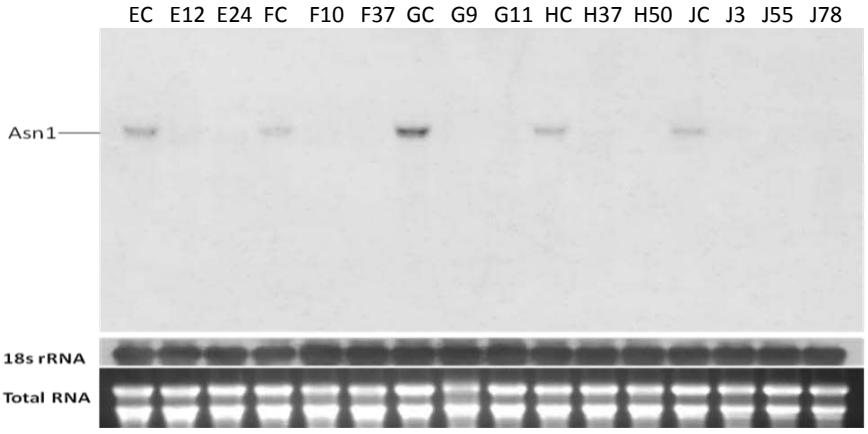
Figure 1. Expression of the *Asn1* Gene in Tubers of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown (**A**) and field-grown (**B**) tuber tissues of events and controls and hybridized with the Asn1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Asn1 transcript is 2.0-kb. The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67 and G9 are not considered for deregulation.

A. Greenhouse-grown tuber



B. Field-grown tubers

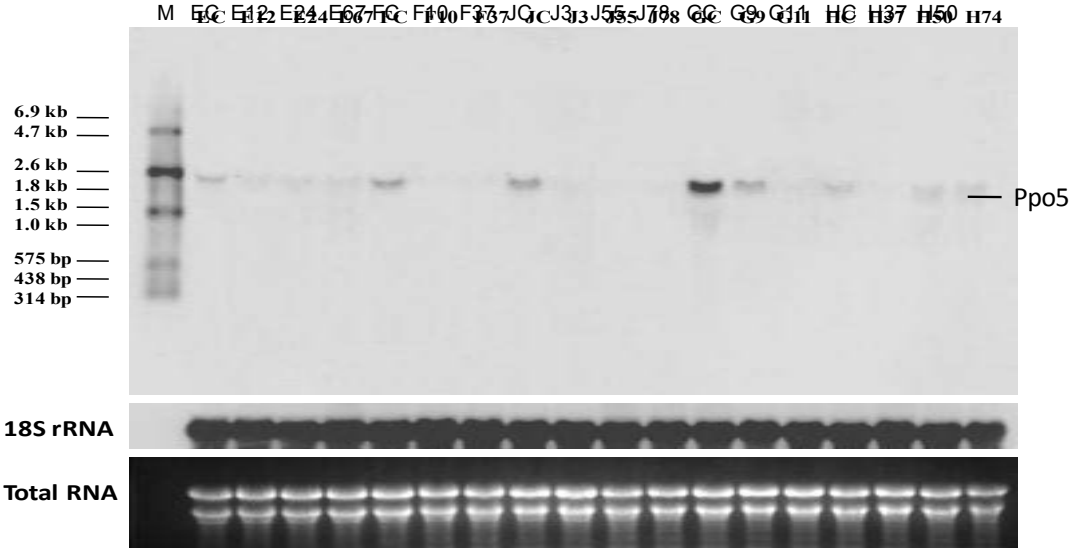


EC= control Russet Burbank, FC = control Ranger Russet, JC = control Atlantic, GC = control G, HC = control H, M = RNA marker

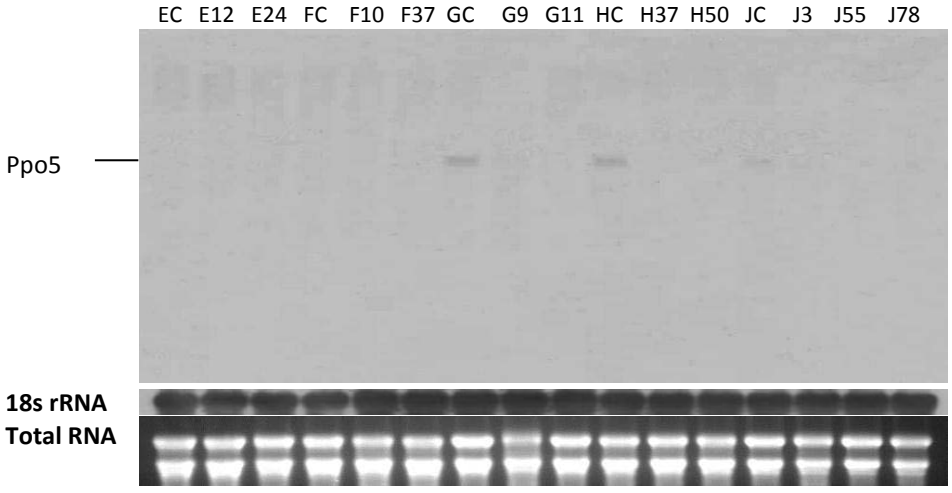
Figure 2. Expression of the *Ppo5* Gene in Tubers of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown (**A**) and field-grown (**B**) tuber tissues of events and controls and hybridized with the Ppo5 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Ppo5 transcript is 1.95-kb (see Genbank Accession U22921). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67 and G9 are not considered for deregulation.

A. Greenhouse-grown tubers



B. Field grown-tubers

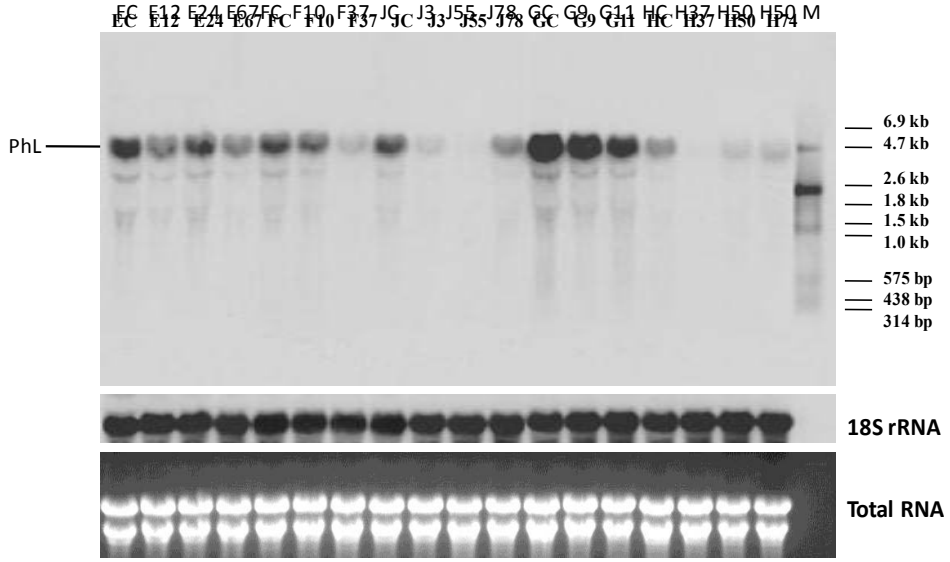


EC= control Russet Burbank, FC = control Ranger Russet, JC = control Atlantic, GC = control G, HC = control H, M = RNA marker

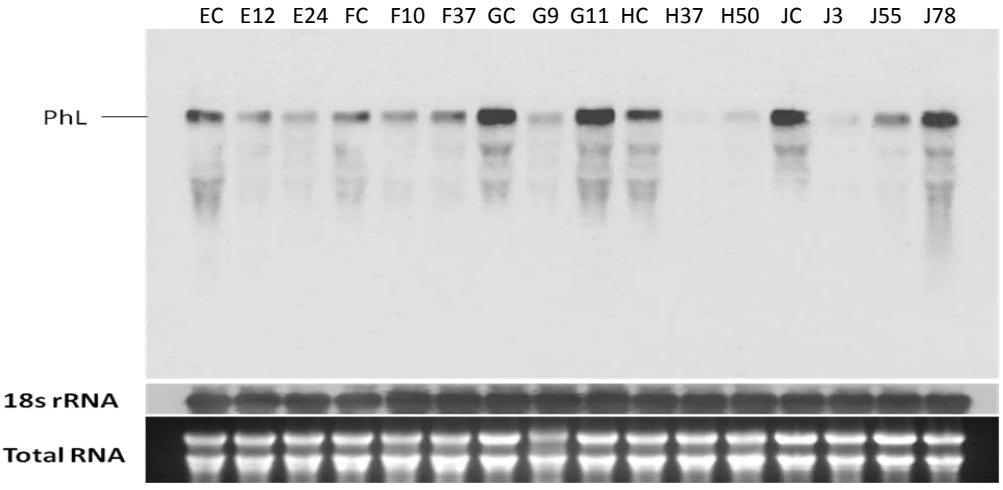
Figure 3. Expression of the *PhL* Gene in Tubers of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown (**A**) and field-grown (**B**) tuber tissues of events and controls and hybridized with the PhL probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the PhL transcript is 4.9-kb (see Genbank Accession X52385). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67 and G9 are not considered for deregulation.

A. Greenhouse-grown tubers



B. Field grown-tubers

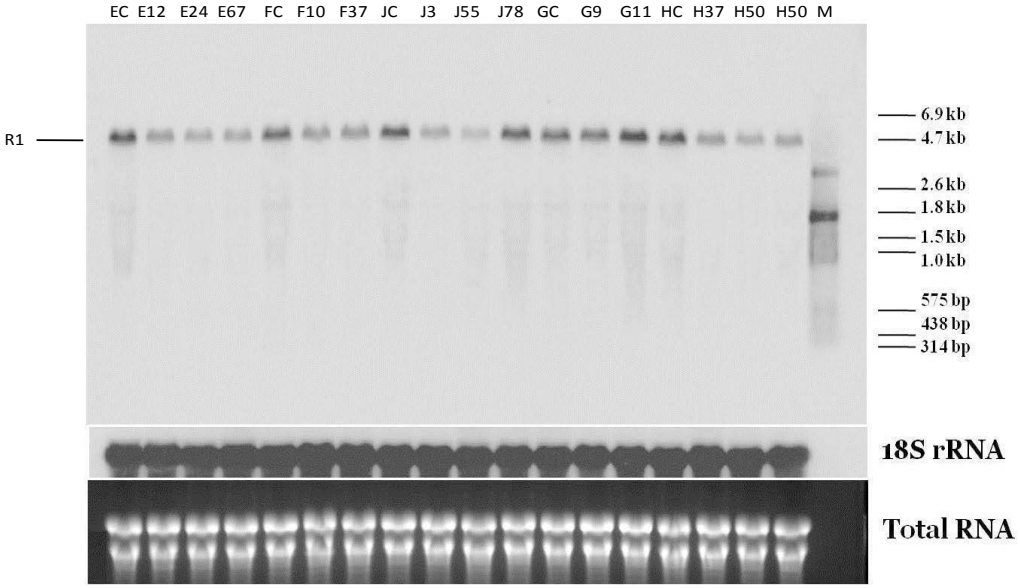


EC= control Russet Burbank, FC = control Ranger Russet, JC = control Atlantic, GC = control G, HC = control H, M = RNA marker

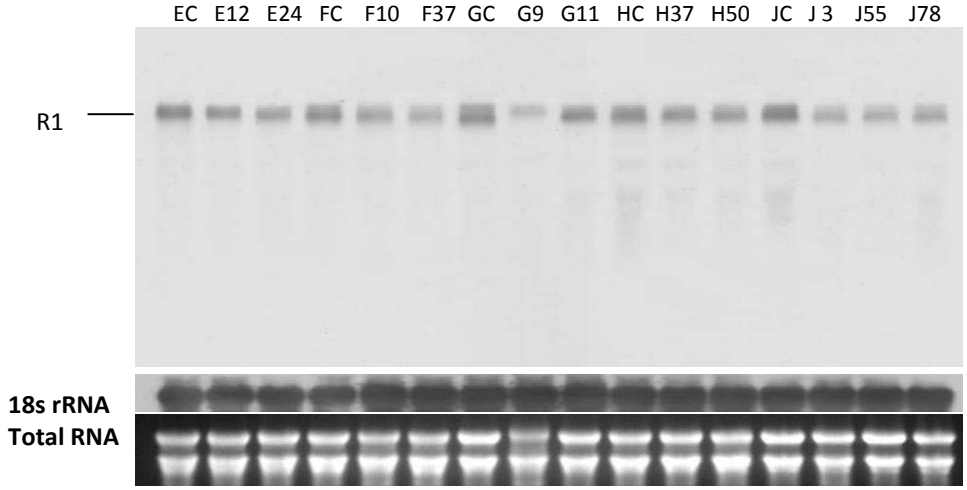
Figure 4. Expression of the *R1* Gene in Tubers of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown (**A**) and field-grown (**B**) tuber tissues of events and controls and hybridized with the R1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the R1 transcript is 4.8-kb (see Genbank Accession Y09533). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67 and G9 are not considered for deregulation.

A. Greenhouse-grown tubers



B. Field grown-Tubers



EC= control Russet Burbank, FC = control Ranger Russet, JC = control Atlantic, GC = control G, HC = control H, M = RNA marker

Target Gene Expression in Stolons

As shown in **Figures 5, 6, 7, and 8**, the *Asn1*, *Ppo5*, and *R1* genes were expressed at low levels in stolons of untransformed plants. In contrast, high transcript levels in the controls were associated with the *PhL* gene.

The transcript levels for the *Asn1* gene were lower in stolons from all events, although slight for H37, compared with control stolons from greenhouse-grown plants (**Figure 5**). This modification was expected and is not linked to any undesired or unanticipated new phenotype.

Transcript levels of *Ppo5* were lowered in events E24, G11, and H50 (**Figure 6**). For Ranger Russet and Atlantics, transcript levels in controls and events were at such low levels that silencing was not detectable.

Expression of the *PhL* gene was also down-regulated in the stolons of most events (F10, F37, E12, E24, J55, H37, and H50). However, *PhL* gene expression was unchanged for J78 and G11 stolons, which do not contain a functionally-active PhL/R1 promoter silencing cassette (**Figure 7)**. Expression also did not appear to be silenced in stolons of J3.

The amounts of transcript for the *R1* gene were slightly reduced ("whispered") in stolons of most events (**Figure 8**). This molecular change contributed to lowering of reducing sugars in tubers. However, events J78 and G11 lack the PhL/R1 promoter silencing cassette and, consequently, displayed no reductions in transcript levels for the associated genes in stolons.

Figure 5. Expression of the *Asn1* Gene in Stolon Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Asn1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Asn1 transcript is 2.0-kb. The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.

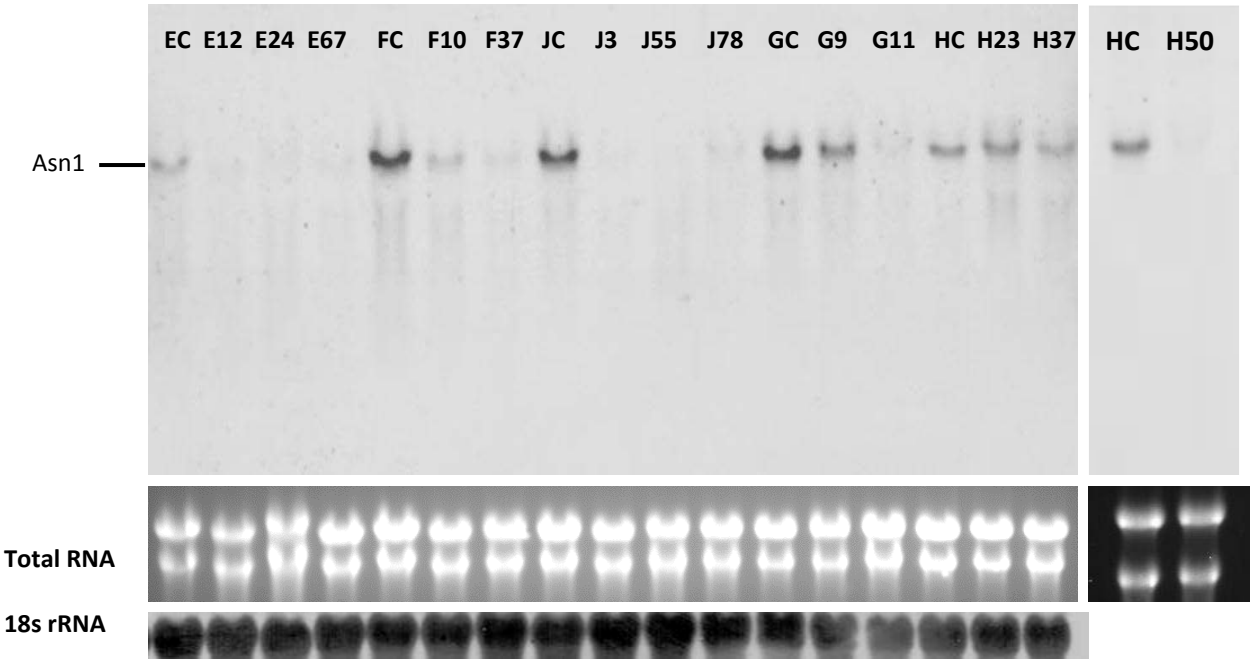


Figure 6. Expression of the *Ppo5* Gene in Stolon Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Ppo5 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Ppo5 transcript is 1.95-kb (see Genbank Accession U22921). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.

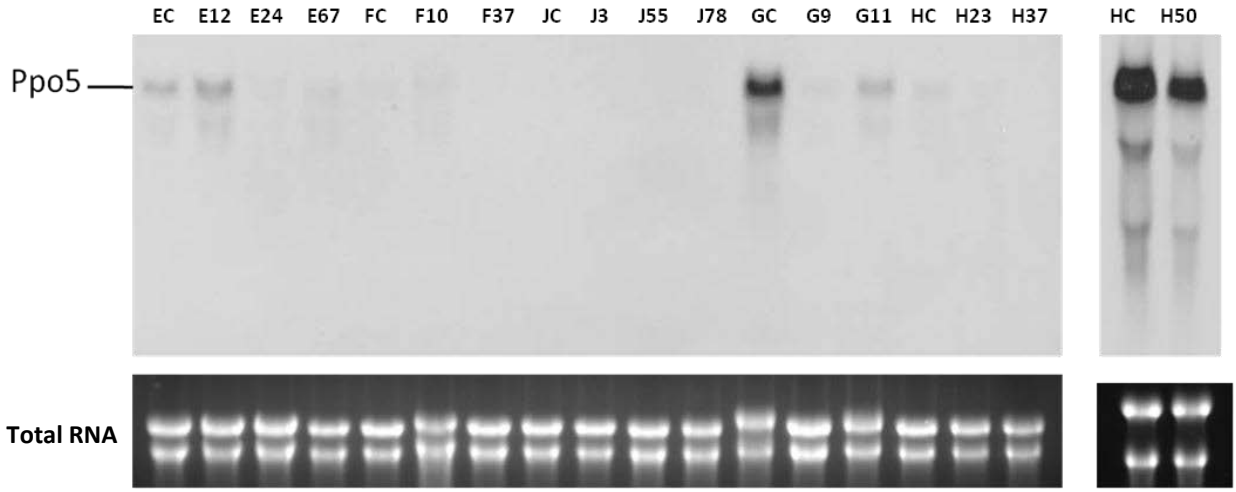


Figure 7. Expression of the *PhL* Gene in Stolon Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the PhL probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the PhL transcript is 4.9-kb (see Genbank Accession X52385). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67 and G9 are not considered for deregulation. Event H50 was processed separately with an H control.

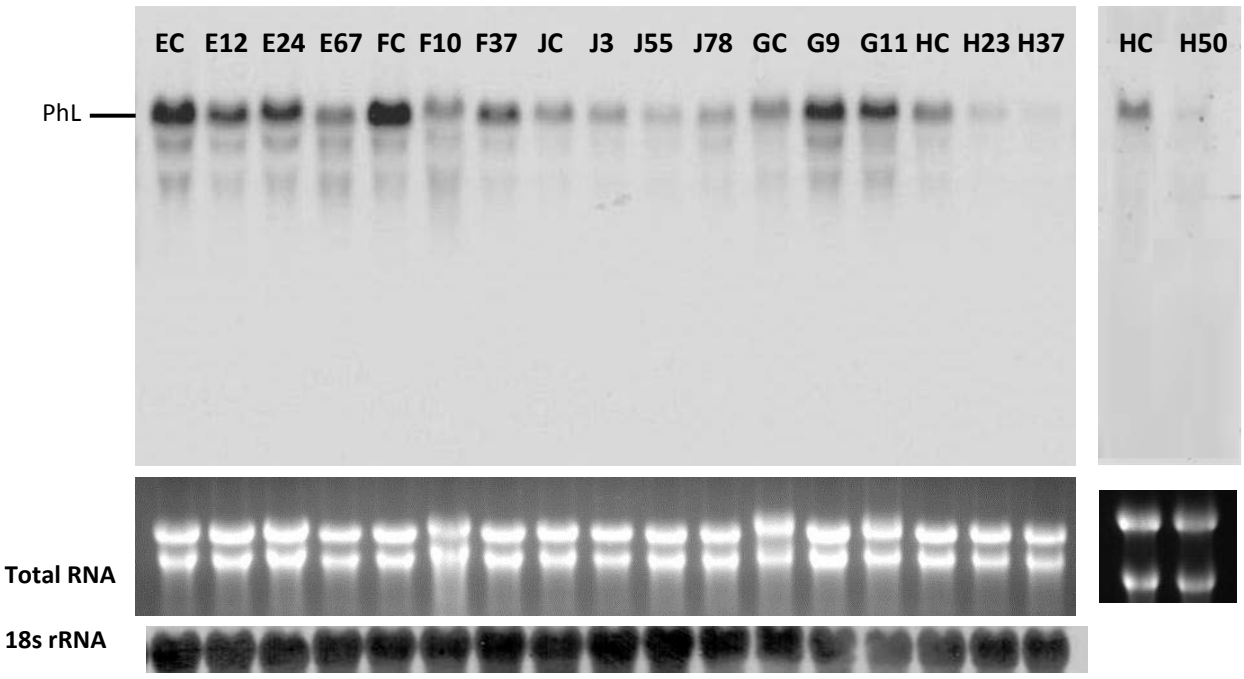
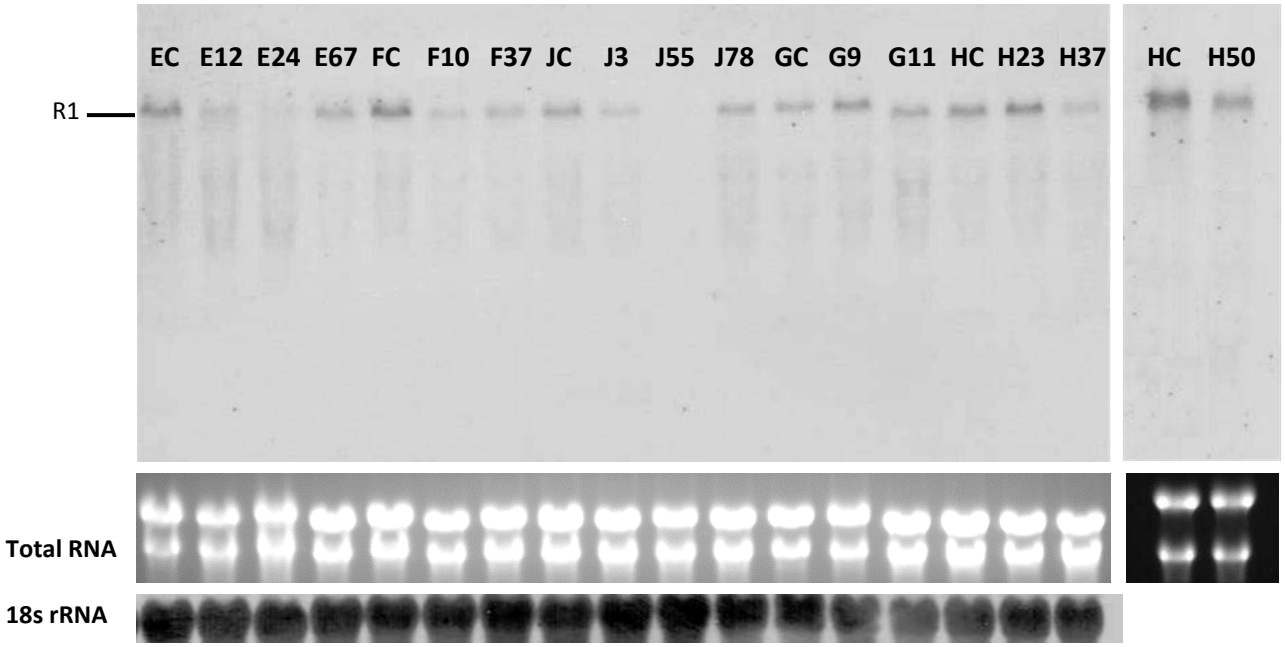


Figure 8. Expression of the *R1* Gene in Stolon Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the R1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the R1 transcript is 4.8-kb (see Genbank Accession Y09533). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.



Transcript Levels in Other Tissues

The Northern expression data for tissues other than tubers and stolons are summarized as below:

(1) In leaf tissues, transcript levels for the *Asn1* gene were similar for the events and their untransformed counterparts, except for F10, which contained lower levels than Ranger Russet **(Figure 9)**. Transcript levels for the *Ppo5* gene expression were in all cases undetectable under our experimental conditions (**Figure 10**). This was expected because the *Ppo5* gene would be most active in tubers and expression was not expected in leaves. Levels for the *PhL* gene were consistently high among the original varieties and their transformed derivatives (**Figure 11**). Transcript levels for the *R1* gene were unaltered in most events when compared to their controls, except for G11, H37, and H50, which contained lower levels than their respective G and H controls (**Figure 12**).

(2) In stem tissues, *Asn1* gene transcript levels were similar for most events and their controls, except that F37, E24, J3, and H50 contained less transcript than the control varieties (**Figure 13**). Interestingly, transcript levels for the *Ppo5* gene were reduced in all events submitted for deregulation, except for G11 (**Figure 14**). *PhL* gene expression was similar in all events and their controls (**Figure 15**), and *R1* gene expression was reduced in G11 only **(Figure 16)**.

(3) In root tissues, transcript levels for the *Asn1* gene were reduced in six events (F10, F37, E12, J3, J55, and H50), and remained unchanged for E24, J78, G11, and H37 (**Figure 17**). Transcript levels for the *Ppo5* gene were reduced in F10, F37, E12, H37, and H50 (**Figure 18**). This indicates that the promoters used to drive silencing are partially functional in underground tissues. In Atlantic and G varieties, low levels of transcripts were observed in both controls and events, suggesting that *Ppo5* gene activity may be naturally lower in roots of those varieties. No significant reductions in expression levels were observed for the *PhL* gene, and for the *R1* gene F10, F37, and H50 were lower (**Figures 19 and 20**).

(4) In floral tissues, *Asn1* gene transcript levels were lower for the 8 events (the H variety does not flower, so no data were obtained for H37 and H50) than for the original varieties (**Figure 21**). Transcripts were not detectable for the *Ppo5* gene (**Figure 22**). Expression levels of the *PhL* gene for all events were not reduced compared with their untransformed controls **(Figure 23)**. For the *R1* gene, expression levels in F37 were lower than the Ranger Russet control, but all other events were similar to controls **(Figure 24)**.

It can be concluded that transcript levels of the four targeted genes were occasionally down-regulated in tissues other than tubers and stolons. In these cases, the reductions were partial and were not associated with agronomic differences in the events when compared to untransformed controls in field trials.

Target Gene Expression in Leaf Tissue

Tran**script levels in** leaf tissues were determined by using greenhouse-grown plants, assuming that performance in the greenhouse will predict what occurs in the field. Indeed, there is extensive evidence in the scientific literature (Anand *et al*. 2003; Kuipers *et al*. 1992; Mikschofsky *et al*. 2011) to support that hypothesis.

In leaf tissues, transcript levels for the *Asn1* gene were similar for the events and their untransformed counterparts, except for F10, which contained lower levels than Ranger Russet **(Figure 9)**. Transcript levels for the *Ppo5* gene expression were in all cases undetectable under our experimental conditions (**Figure 10**). This was expected because the *Ppo5* gene would be most active in tubers and expression was not expected in leaves. Levels for the *PhL* gene were consistently high among the original varieties and their transformed derivatives (**Figure 11**). Transcript levels for the *R1* gene were unaltered in most events when compared to their controls, except for G11, H37, and H50, which contained lower levels than their respective G and H controls (**Figure 12**).

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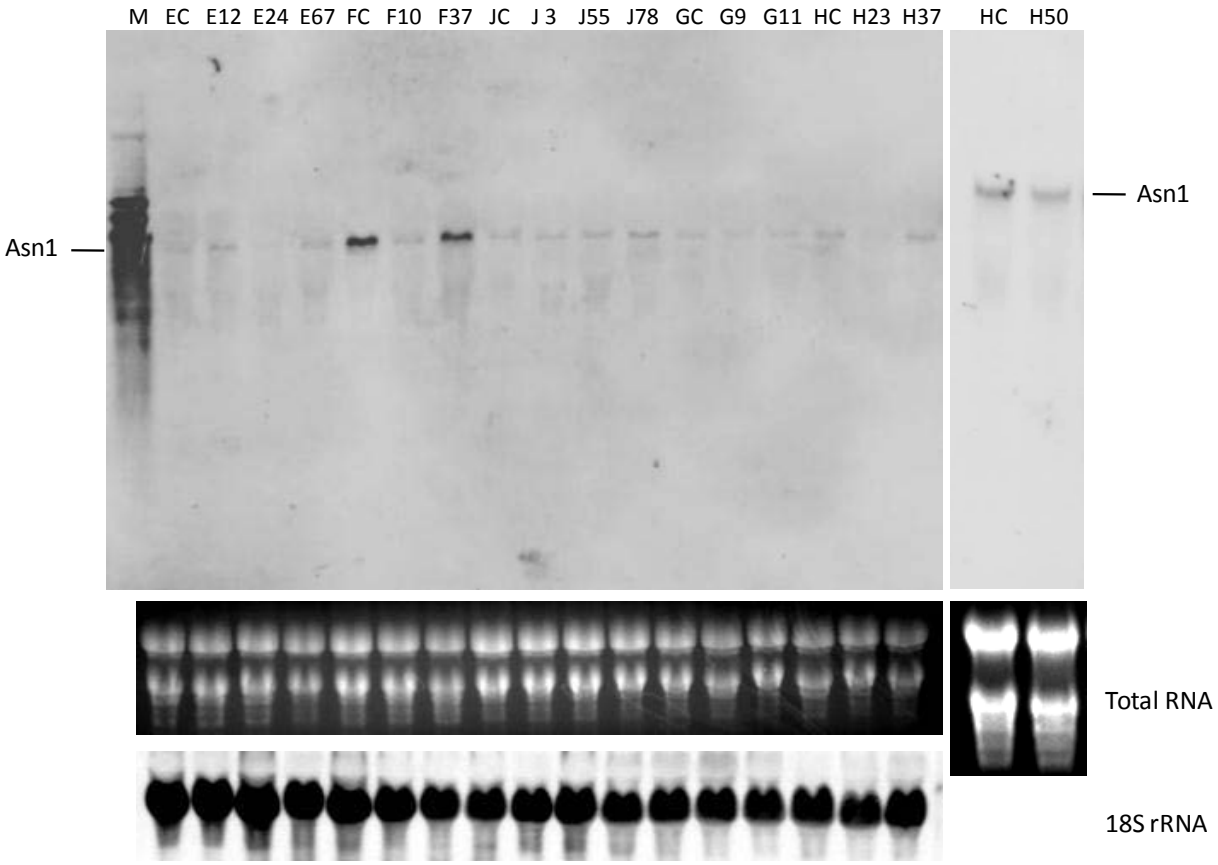
Figure 9. Expression of the *Asn1* Gene in Leaf Tissue of Events and Controls

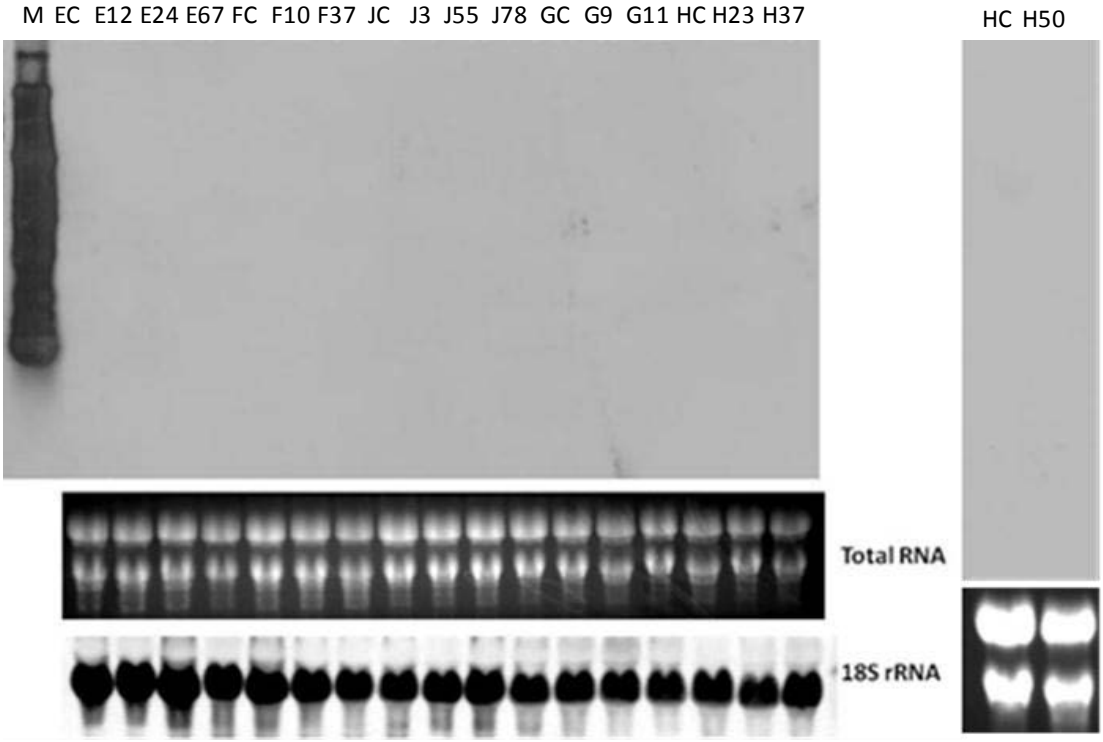
Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Asn1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Asn1 transcript is 2.0-kb. The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.



EC= control Russet Burbank, FC = control Ranger Russet, JC = control Atlantic, GC = control G, HC = control H, M = RNA marker

Figure 10. Expression of the *Ppo5* Gene in Leaf Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Ppo5 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Ppo5 transcript is 1.95-kb (see Genbank Accession U22921). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.



EC= control Russet Burbank, FC = control Ranger Russet, JC = control Atlantic, GC = control G, HC = control H, M = RNA marker

Figure 11. Expression of the *PhL* Gene in Leaf Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the PhL probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the PhL transcript is 4.9-kb (see Genbank Accession X52385). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9, and H23 are not considered for deregulation. Event H50 was processed separately with an H control.

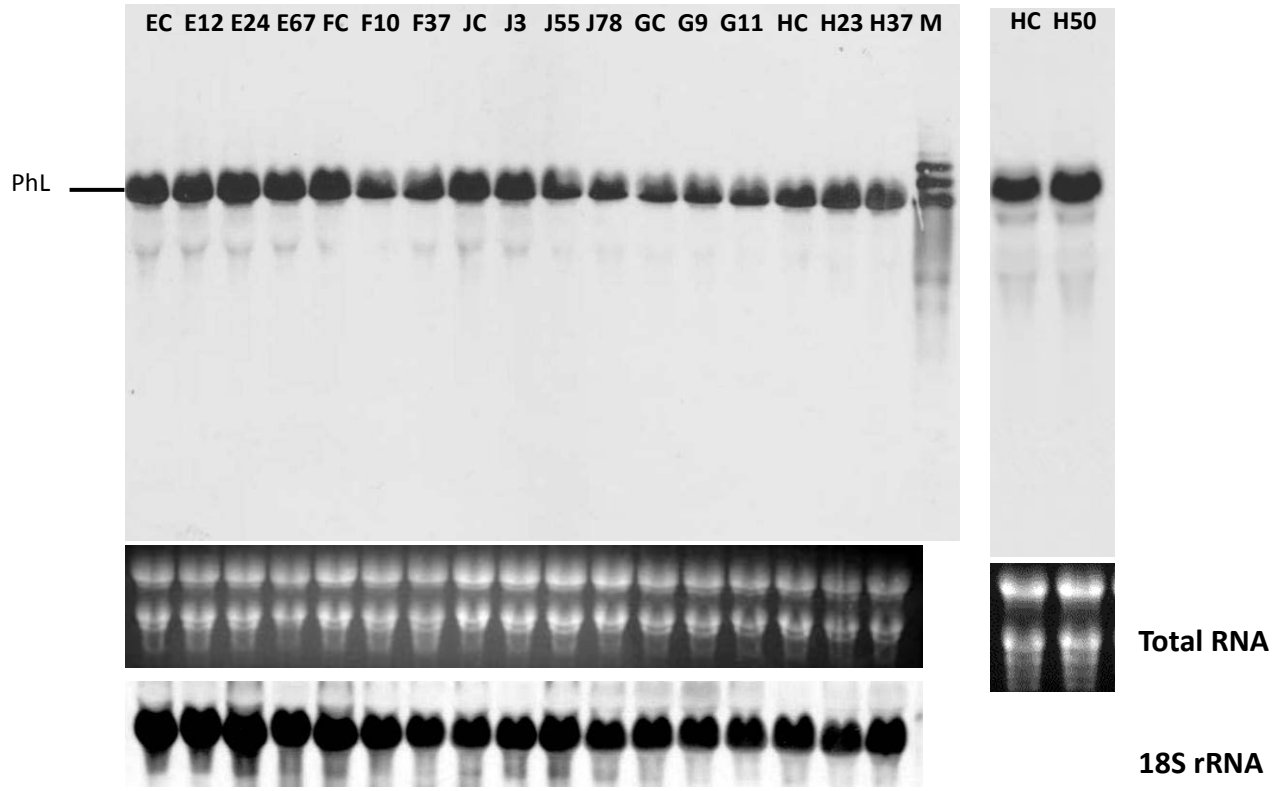
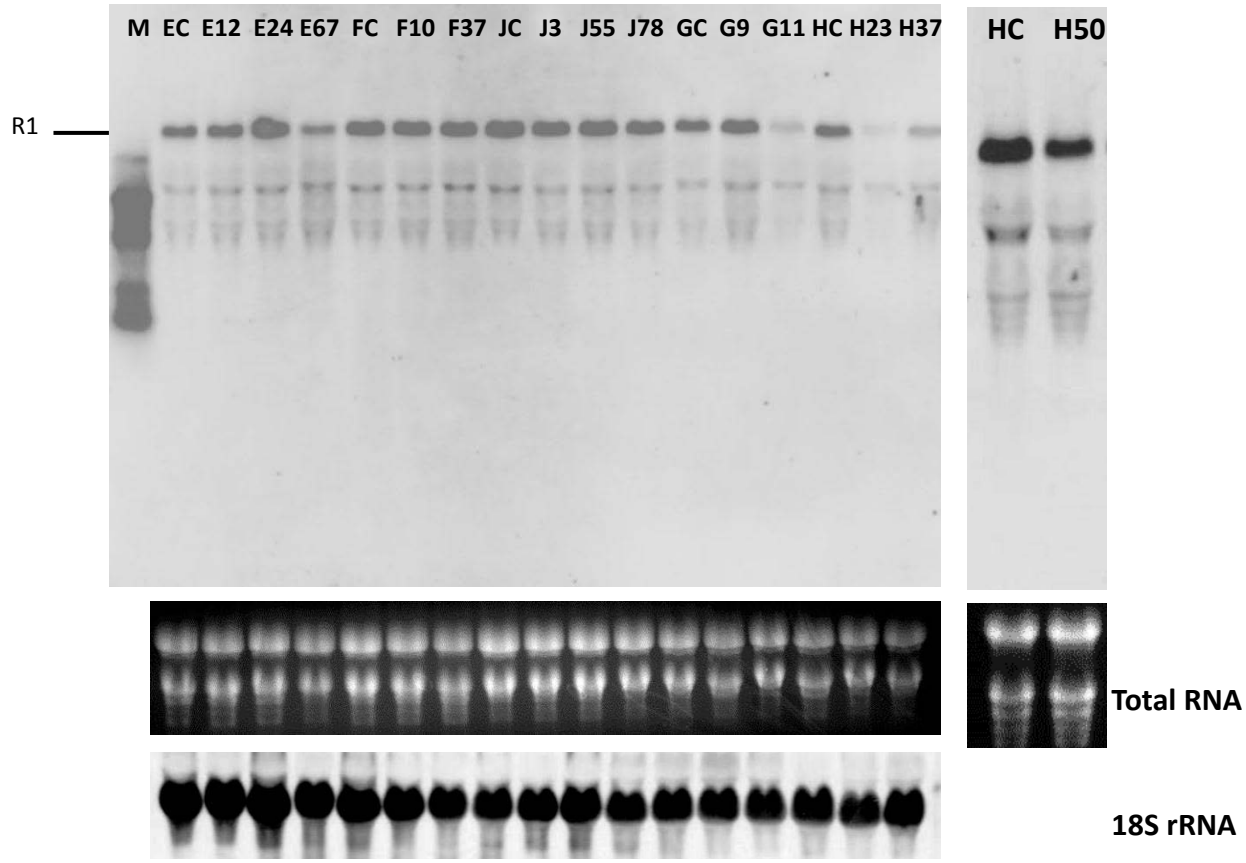


Figure 12. Expression of the *R1* Gene in Leaf Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the R1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the R1 transcript is 4.8-kb (see Genbank Accession Y09533). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.



EC= control Russet Burbank, FC = control Ranger Russet, JC = control Atlantic, GC = control G, HC = control H, M = RNA marker

Target Gene Expression in Stems

In stem tissues, *Asn1* gene transcript levels were similar for most events and their controls, except that F37, E24, J3, and H50 contained less transcript than the control varieties (**Figure 13**). Interestingly, transcript levels for the *Ppo5* gene were reduced in all events submitted for deregulation, except for G11 (**Figure 14**). *PhL* gene expression was similar in all events and their controls (**Figure 15**), and *R1* gene expression was reduced in G11 only **(Figure 16)**.

Figure 13. Expression of the *Asn1* Gene in Stem Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Asn1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Asn1 transcript is 2.0-kb. The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.

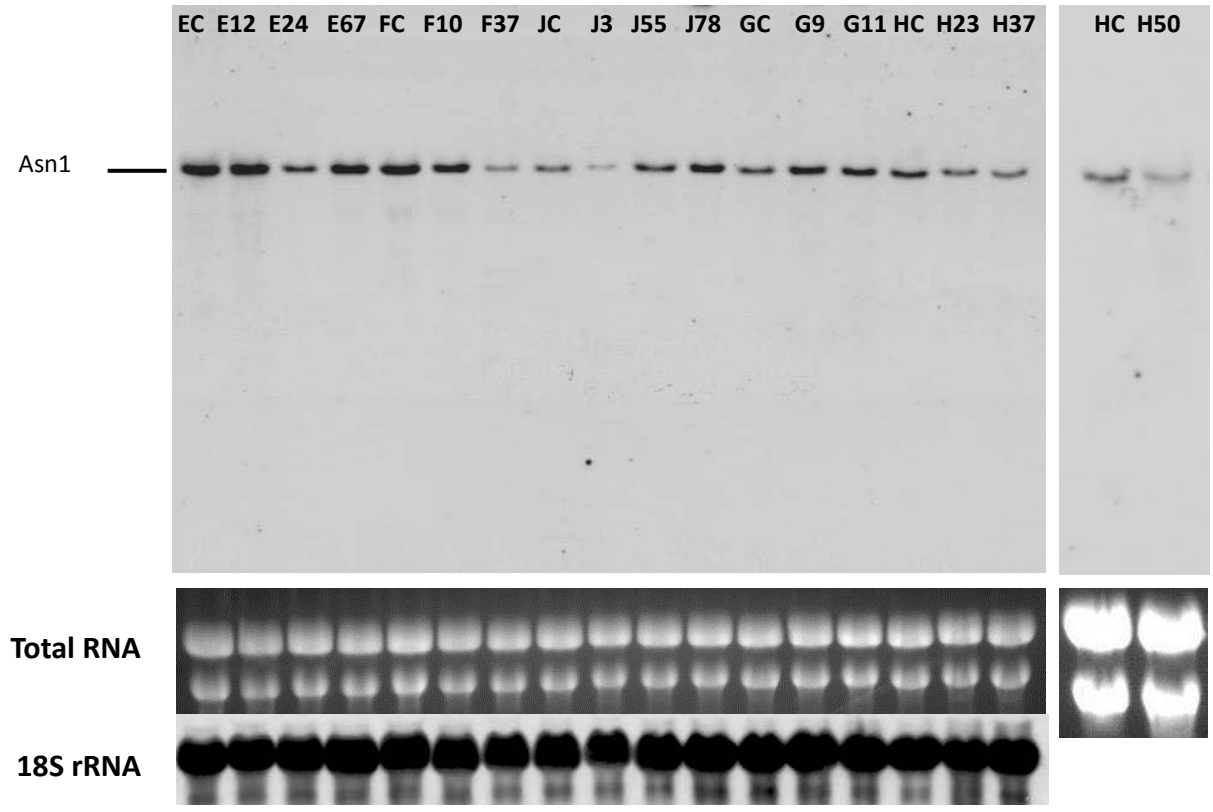
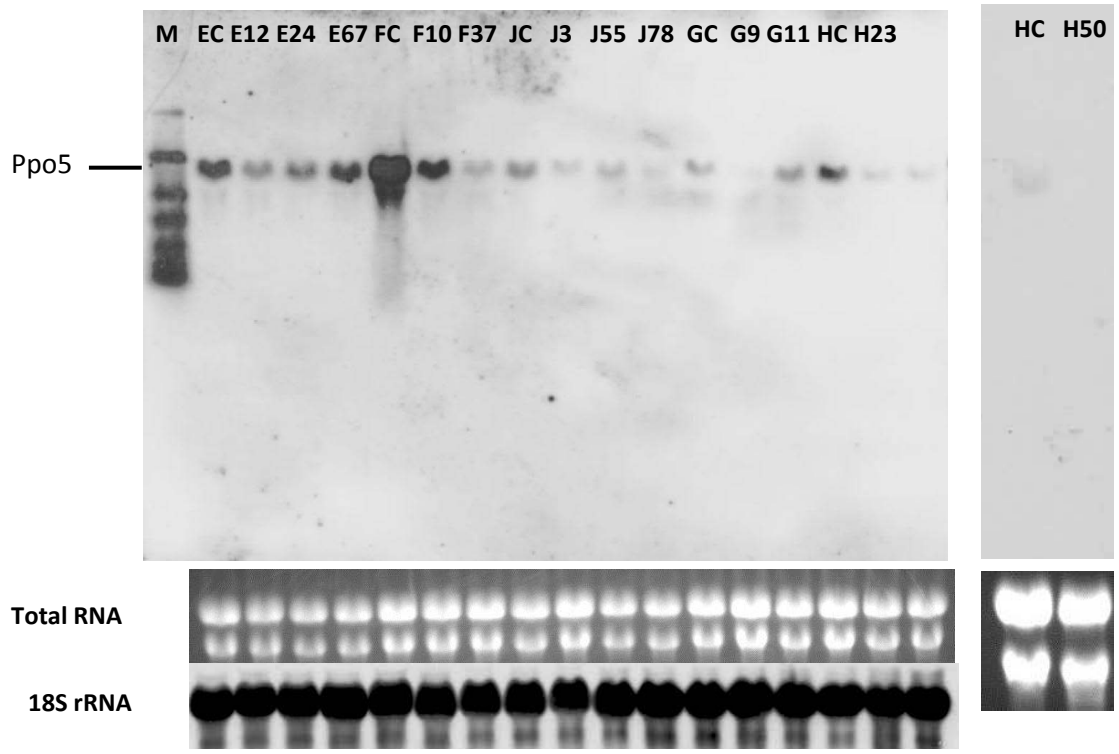


Figure 14. Expression of the *Ppo5* Gene in Stem Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Ppo5 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Ppo5 transcript is 1.95-kb (see Genbank Accession U22921). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.



EC= control Russet Burbank, FC = control Ranger Russet, JC = control Atlantic, GC = control G, HC = control H, M = RNA marker

Figure 15. Expression of the *PhL* Gene in Stem Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the PhL probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the PhL transcript is 4.9-kb (see Genbank Accession X52385). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.

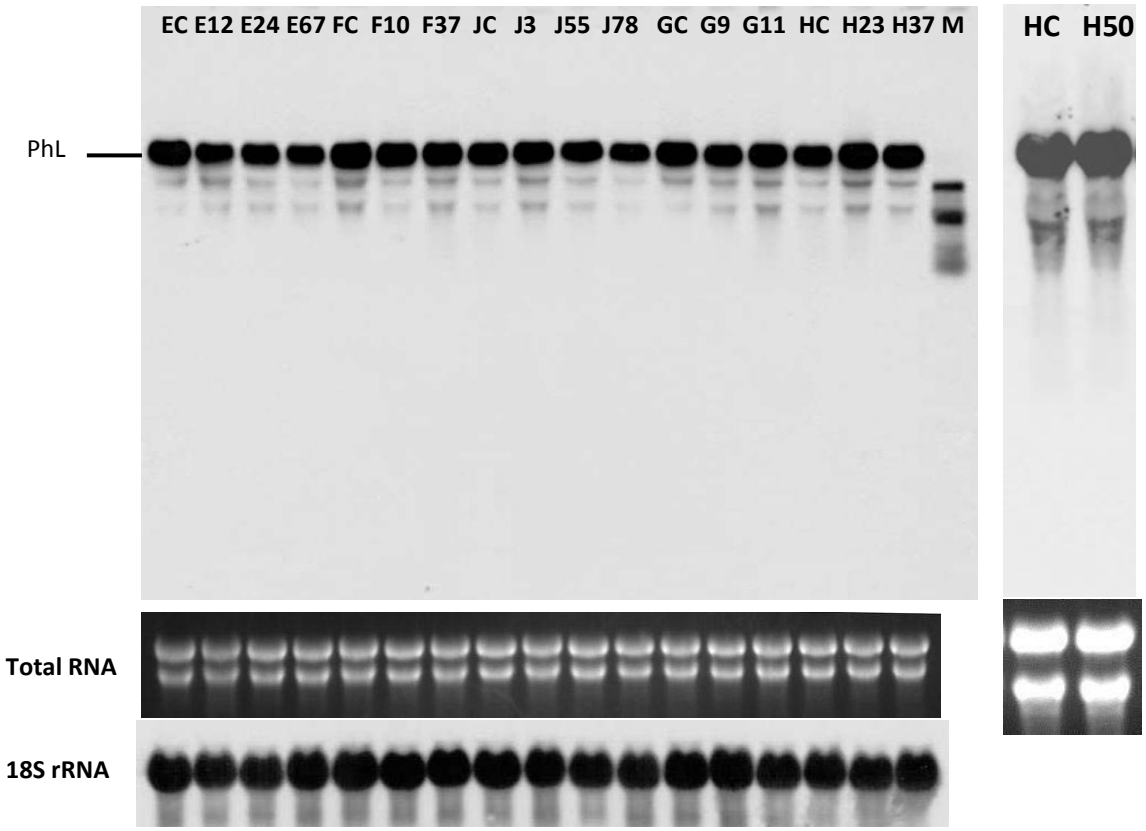
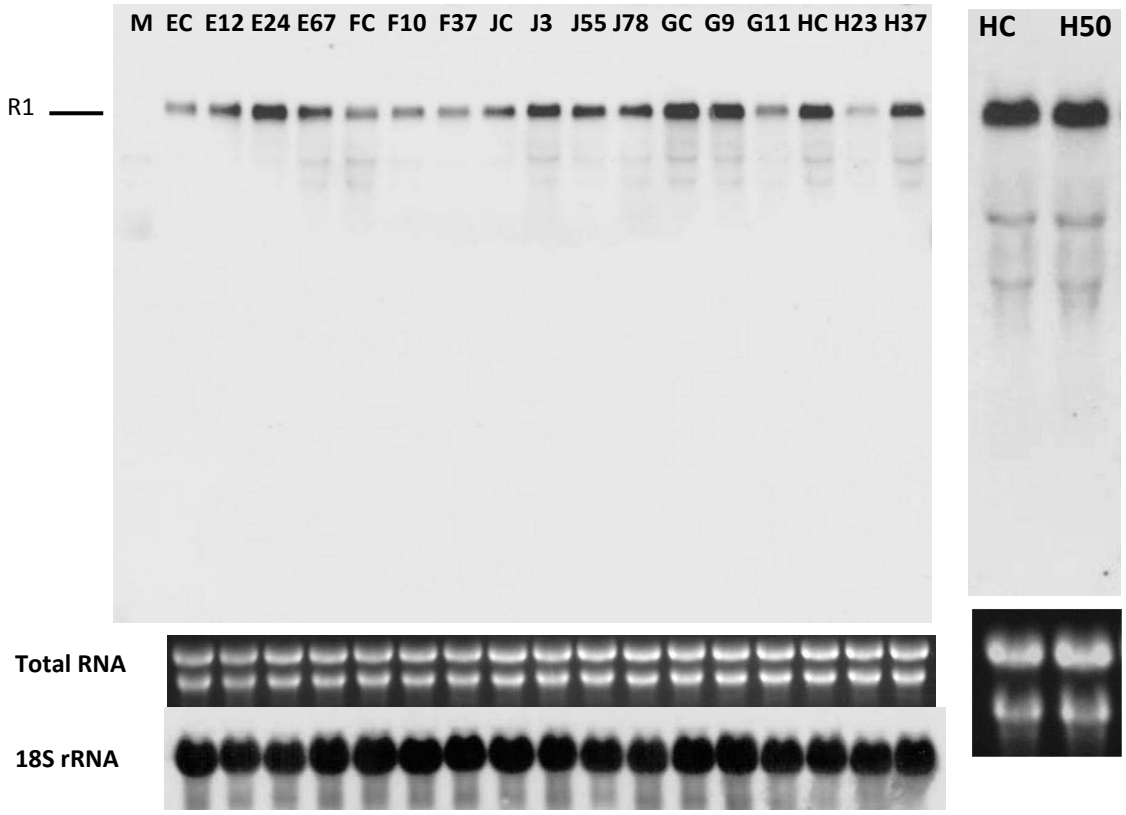


Figure 16. Expression of the *R1* Gene in Stem Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the R1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the R1 transcript is 4.8-kb (see Genbank Accession Y09533). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.



EC= control Russet Burbank, FC = control Ranger Russet, JC = control Atlantic, GC = control G, HC = control H, M = RNA marker

Target Gene Expression in Roots

In root tissues, transcript levels for the *Asn1* gene were reduced in six events (F10, F37, E12, J3, J55, and H50), and remained unchanged for E24, J78, G11, and H37 (**Figure 17**). Transcript levels for the *Ppo5* gene were reduced in F10, F37, E12, H37, and H50 (**Figure 18**). This indicates that the promoters used to drive silencing are partially functional in underground tissues. In Atlantic and G varieties, low levels of transcripts were observed in both controls and events, suggesting that *Ppo5* gene activity may be naturally lower in roots of those varieties. No significant reductions in expression levels were observed for the *PhL* gene, and for the *R1* gene F10, F37, and H50 were lower (**Figures 19 and 20**).

Figure 17. Expression of the *Asn1* Gene in Root Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Asn1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Asn1 transcript is 2.0-kb. The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.

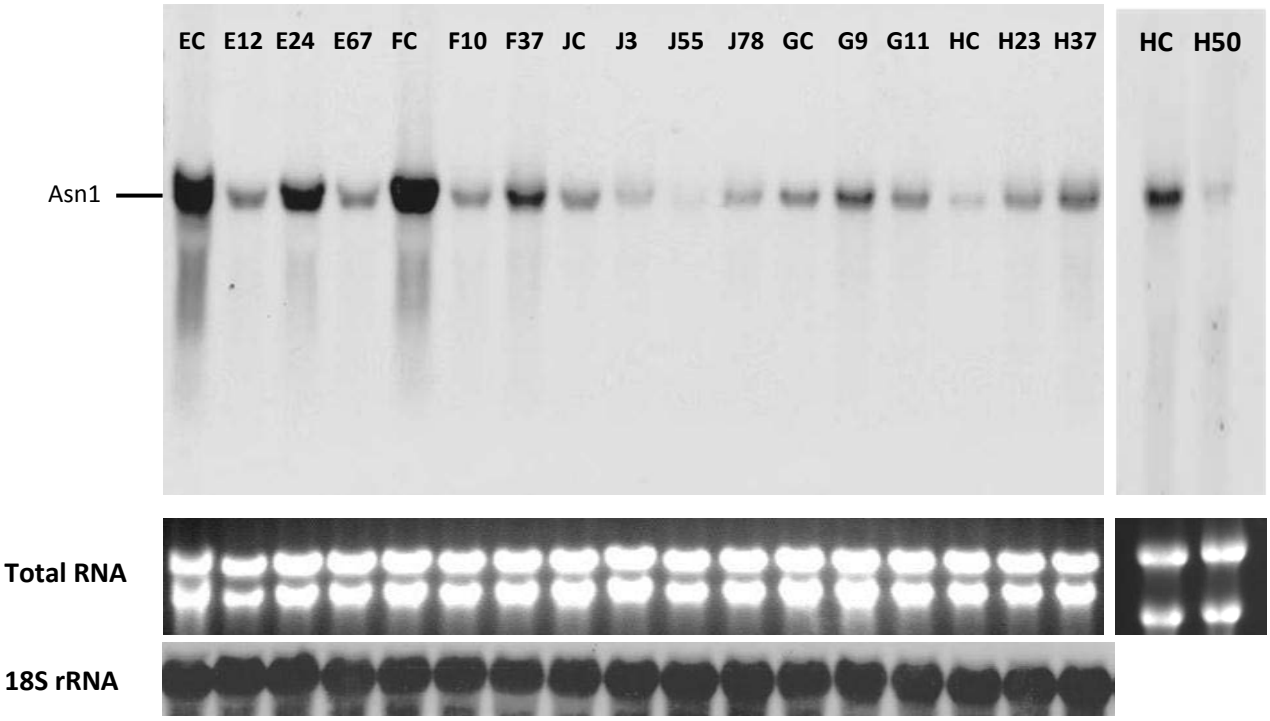


Figure 18. Expression of the *Ppo5* Gene in Root Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Ppo5 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Ppo5 transcript is 1.95-kb (see Genbank Accession U22921). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.

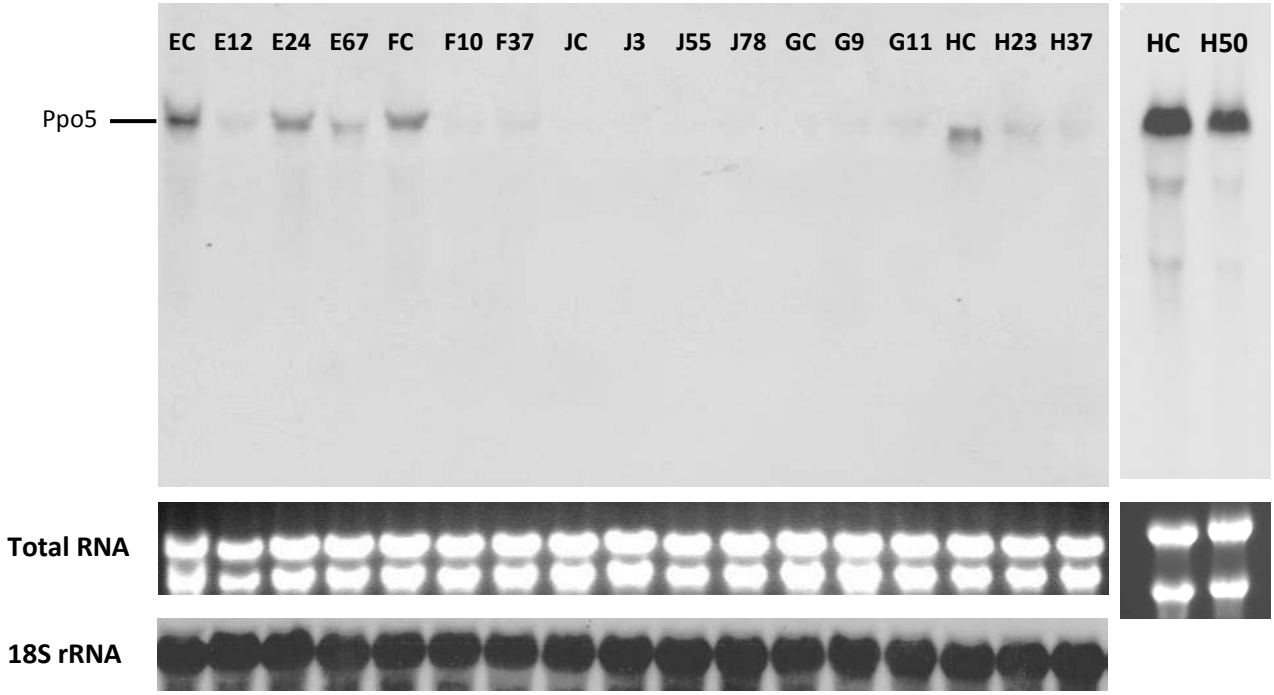


Figure 19. Expression of the *PhL* Gene in Root Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the PhL probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the PhL transcript is 4.9-kb (see Genbank Accession X52385). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.

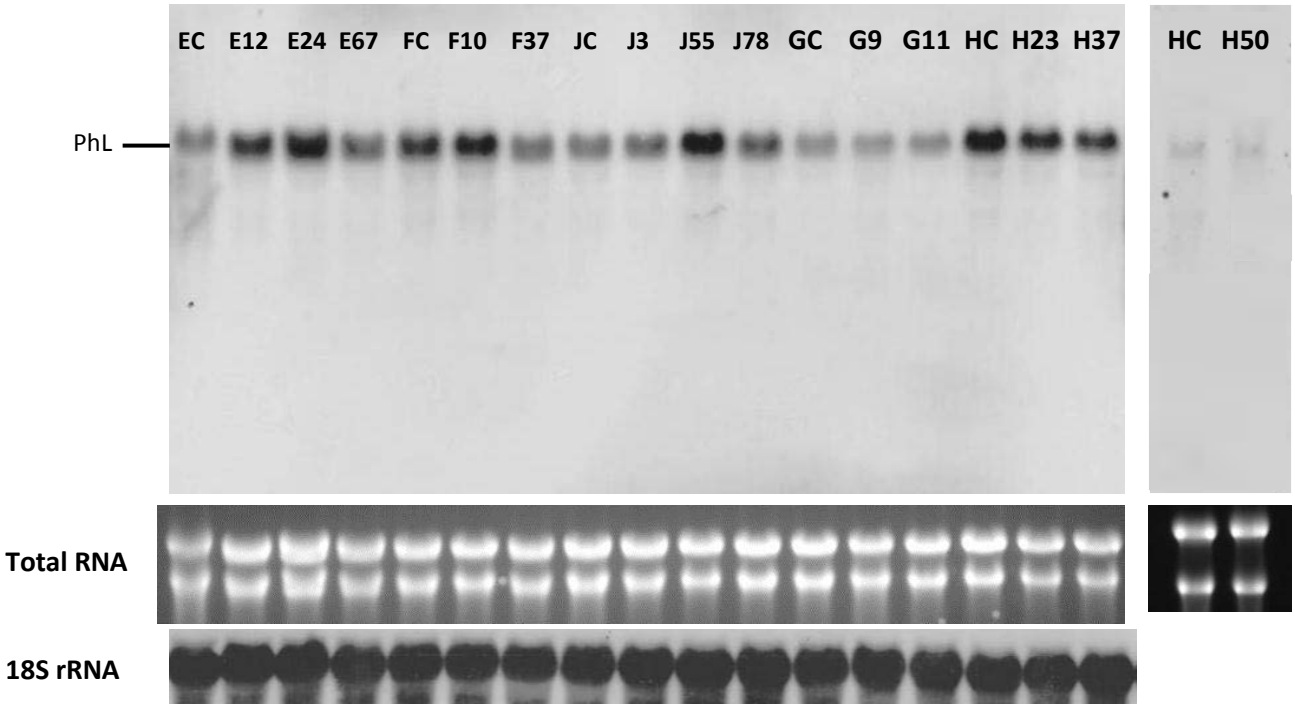
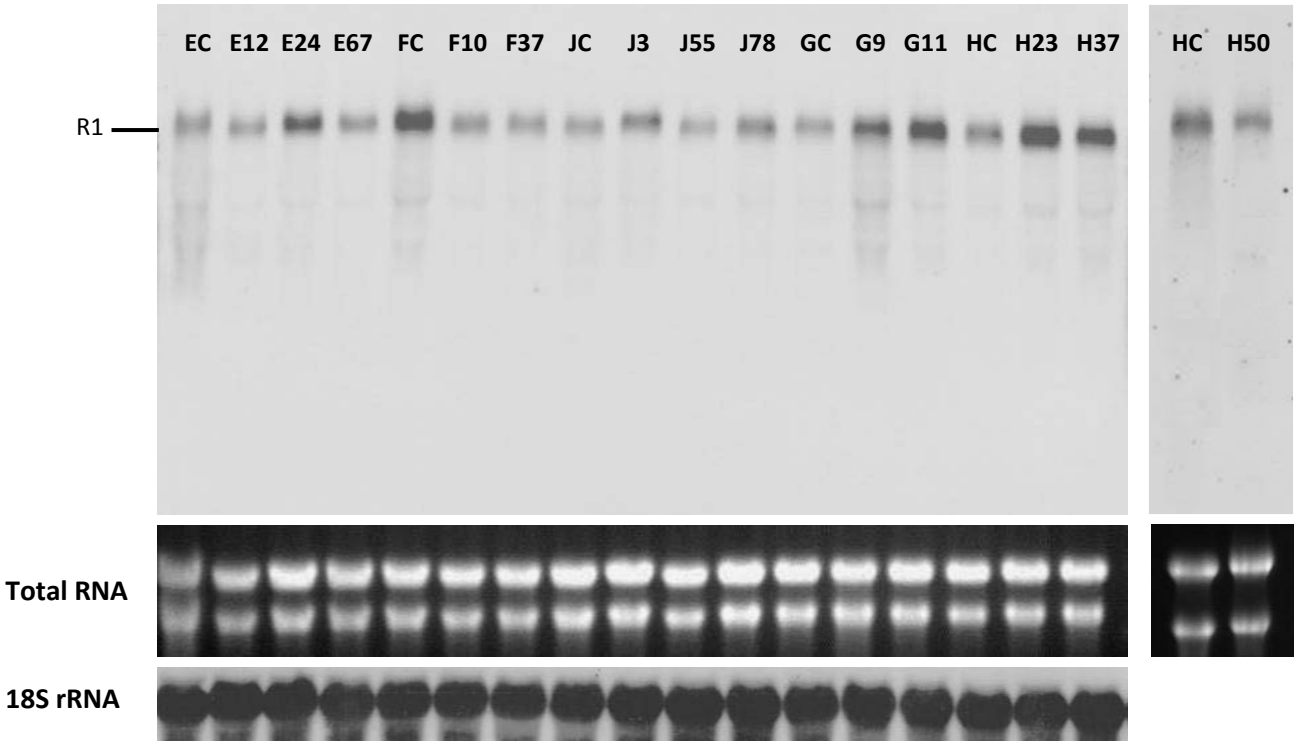


Figure 20. Expression of the *R1* Gene in Root Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the R1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the R1 transcript is 4.8-kb (see Genbank Accession Y09533). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, GC and HC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67, G9 and H23 are not considered for deregulation. Event H50 was processed separately with an H control.



Target Gene Expression in Floral Tissue

In floral tissues, *Asn1* gene transcript levels were lower for the 8 events (the H variety does not flower, so no data were obtained for H37 and H50) than for the original varieties (**Figure 21**). Transcripts were not detectable for the *Ppo5* gene (**Figure 22**). Expression levels of the *PhL* gene for all events were not reduced compared with their untransformed controls **(Figure 23)**. For the *R1* gene, expression levels in F37 were lower than the Ranger Russet control, but all other events were similar to controls **(Figure 24)**.

Figure 21. Expression of the *Asn1* Gene in Floral Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Asn1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Asn1 transcript is 2.0-kb. The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, and GC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67 and G9 are not considered for deregulation.

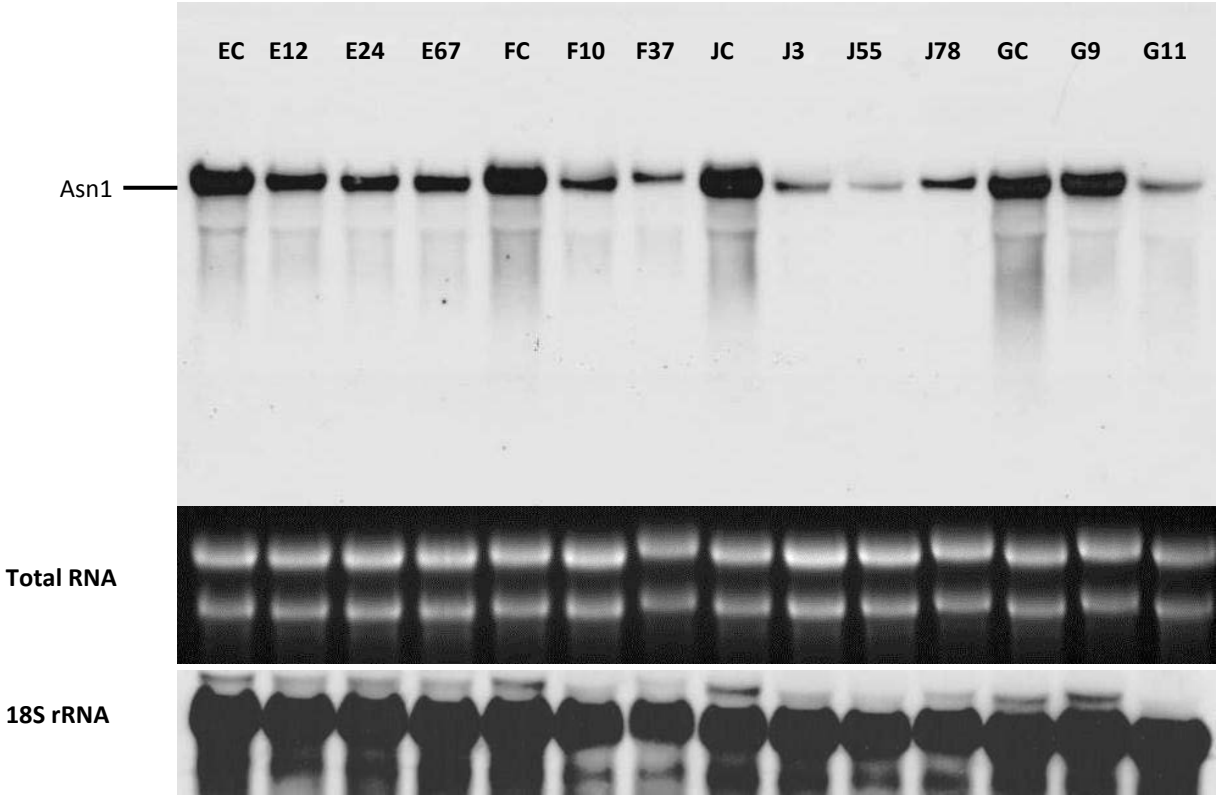


Figure 22. Expression of the *Ppo5* Gene in Floral Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the Ppo5 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the Ppo5 transcript is 1.95-kb (see Genbank Accession U22921). The lower panel shows the amo unts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, and GC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67 and G9 are not considered for deregulation.

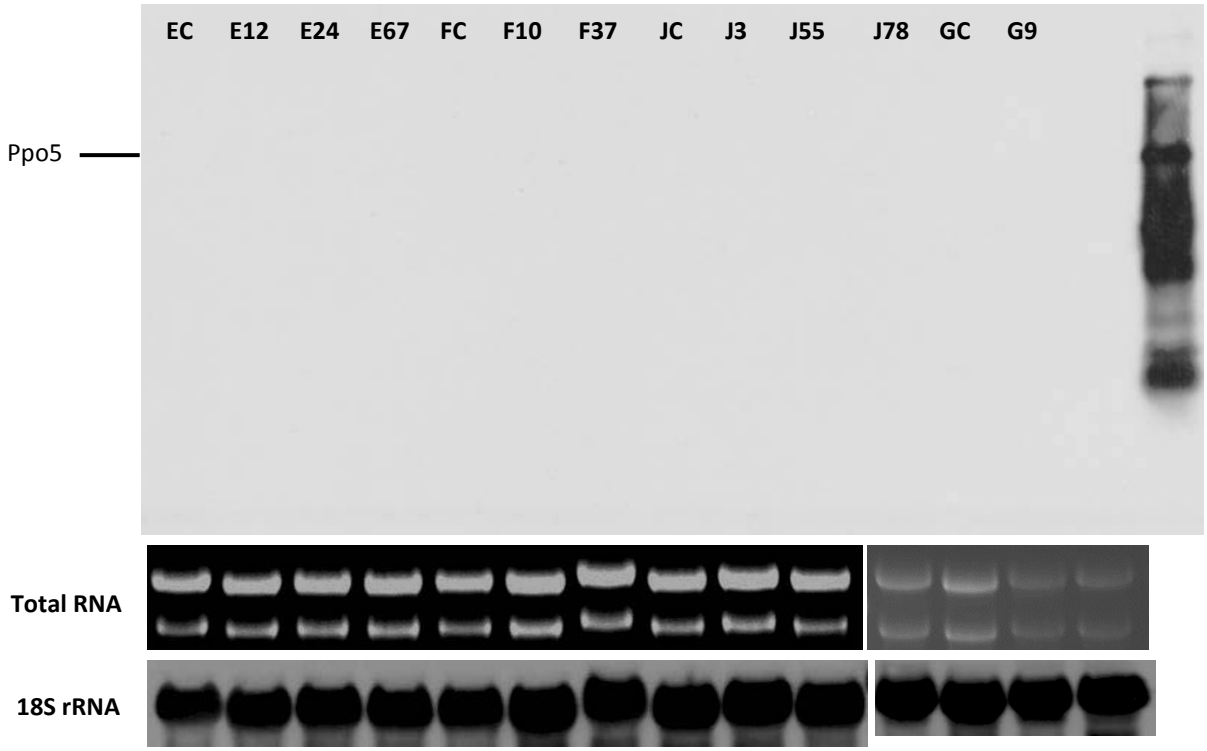


Figure 23. Expression of the *PhL* Gene in Floral Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the PhL probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the PhL transcript is 4.9-kb (see Genbank Accession X52385). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, and GC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67 and G9 are not considered for deregulation.

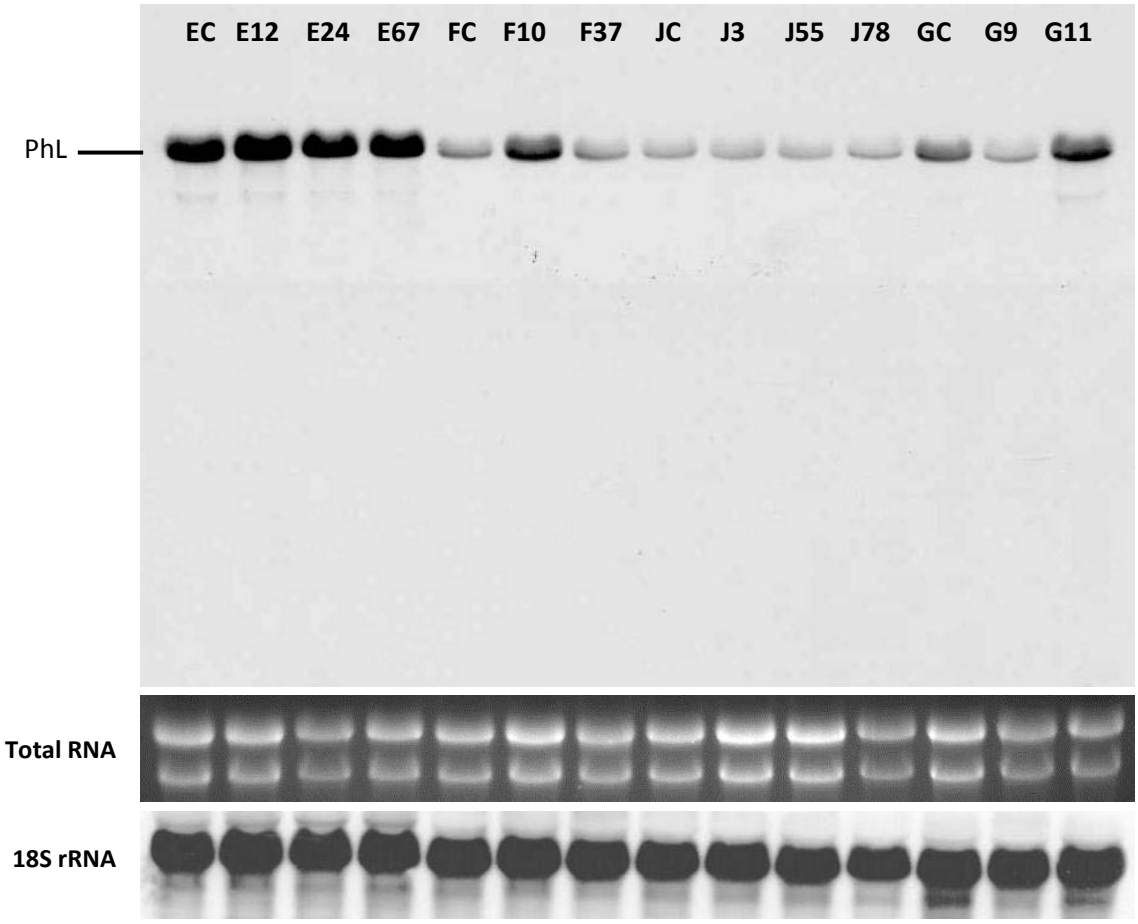
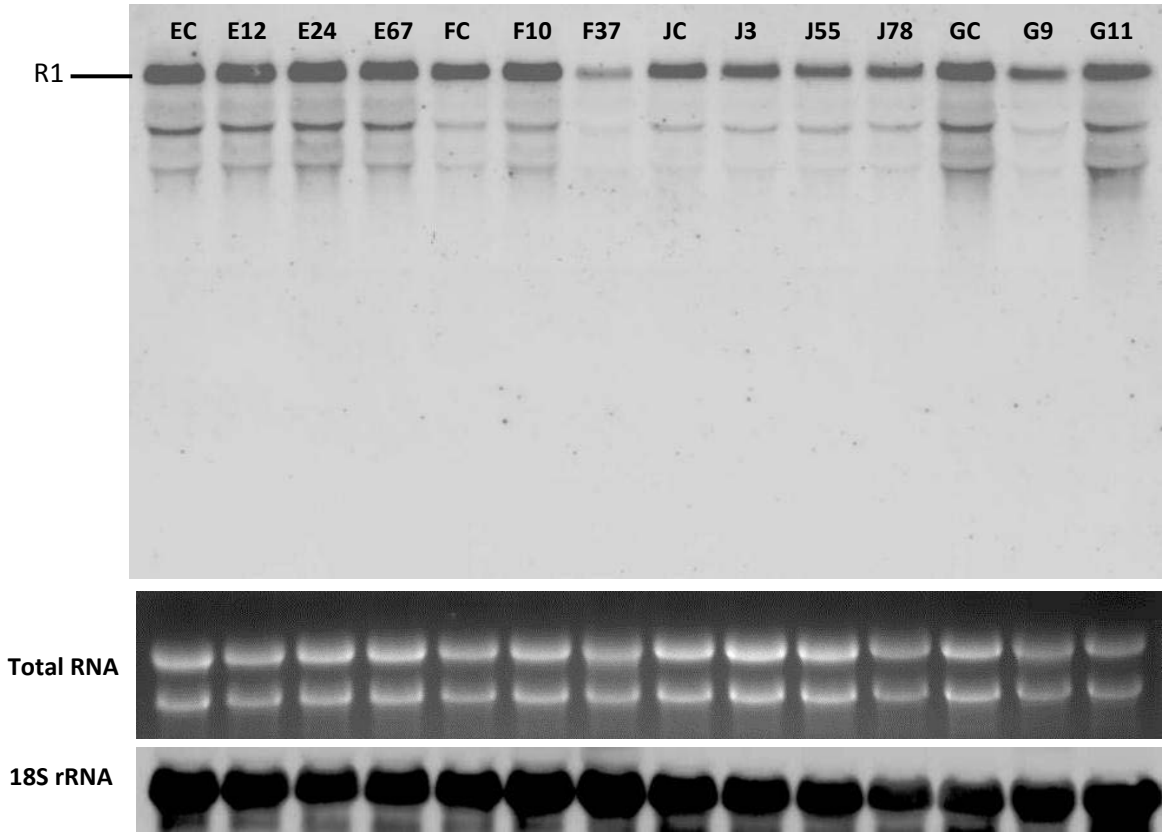


Figure 24. Expression of the *R1* Gene in Floral Tissue of Events and Controls

Total RNA (20 µg) was isolated from greenhouse-grown tissues of events and controls and hybridized with the R1 probe (upper panel) and the internal benchmark 18s rRNA probe (middle panel). The predicted size of the R1 transcript is 4.8-kb (see Genbank Accession Y09533). The lower panel shows the amounts of total RNA as visualized with ethidium bromide (EB). EC, FC, JC, and GC are control samples from the untransformed conventional varieties. Individual event samples are labeled above each lane. The events E67 and G9 are not considered for deregulation.



SUMMARY

It was concluded that transcript levels of the four targeted genes were mostly down-regulated in tubers of the events that carry at least one intact DNA insert, as would be expected from using tuber/stolon-specific promoters. For some events, E24 and H50, all four genes were also down-regulated in stolons. The two events J78 and G11, which contain a truncated DNA insert with a functionally active *Asn1*/*Ppo5* silencing cassette, produced tubers that contained reduced transcript levels for the *Asn1* and *Ppo5* genes only, since they did not contain the *PhL/R1* silencing cassette. Overall, more down-regulation of the four targeted genes occurred in tubers and stolons than in the roots, stems, leaves, or flowers (**Table 1**).

The selected events were more strongly affected in the expression levels of the *Asn1* and *Ppo5* genes than in those of the *R1* and *PhL* genes. This result coincided with our specific interest in (1) preventing the formation of Ppo protein and free ASN to the greatest extent possible, and (2) only partially blocking the conversion of starch into glucose and fructose.

There were occasional changes in transcript levels in tissues other than tubers and stolons, demonstrating some leakiness of the tuber/stolon-specific promoter (**Table 1**). Such event-specific variations may have come about because of position effects. For instance, it is possible that the driving promoters within the DNA insert are enhanced in activity by genetic elements in adjacent plant DNA. An alternative explanation is that small RNAs produced in tubers through expression of the silencing cassettes migrate into other tissues, especially roots and stems (Molnar et al. 2010). In most cases of altered expression levels in tissues other than tubers and stolons, the differences were found to be minor. Unanticipated reductions in gene expression in non-tuber tissues were not associated with agronomic differences in the field and are, therefore, not expected to have any impact on the safety of the plants or their pest potential.

Table 1. Summary of Down-Regulated Genes in Different Tissues

Event	Tubers[1]	Stolons[1]	Roots[1]	Stems[1]	Leaves[1]	Flowers[1, 2]
F10	A P R	A L R	A P R	P	A	A
F37	A P R	A L R	A P R	A P		A R
E12	A P L R	A L R	A P	P		A
E24	A P L R	A P L R		A P		A
J3	A P L R	A R	A	A P		A
J55	A P L R	A L R	A	P		A
J78	A P	A		P		A
G11	A P	A P		R	R	A
H37[3]	A P L R	L R	P	P	R	
H50[3]	A P L R	A P L R	A P R	A P	R	

[1]A = *Asn1*, P = *Ppo*5, L = *PhL*, R = *R1*. Letters in table indicate down-regulated gene expression by tissue.

[2]The partially down-regulated *Asn1* gene expression in flowers might alter the amino acid composition of the event flowers. Such effects will be limited to a reduction in ASN and an increase in GLN. Since ASN and GLN are similar non-essential amino acids, changes in the levels of these compounds is not expected to affect the quality of petal, nectar, and pollen as feed for insects or other organisms.

[3]Events derived from variety H do not produce flowers.

MATERIALS AND METHODS

Plant material. Gene expression levels were determined by carrying out RNA gel blot analyses on tubers, stolons, roots, stems, leaves, and flowers of greenhouse-grown plants of all events and controls. Generation 2 (G2) tubers from the field (Othello, 2010 for Ranger Russet, Russet Burbank and Atlantic, and Rhinelander, 2010 for the G and H varieties) were subjected to this analysis as well. Three replications per event were used in the analysis.

RNA Isolation. RNA was extracted from 1 g of tuber, stolon and root tissue using Plant RNA reagent (Invitrogen, Carlsbad, California), whereas Trizol reagent (Invitrogen) was used to extract RNA from leaves, flowers and stems. The concentration of isolated RNA was measured spectrophotometrically at 260 nm, and RNA quality was confirmed by running a sample on a 1% agarose gel with 1X MOPS buffer (200 mM MOPS, 50 mM NaOAc , 20 mM EDTA , pH7.0) for 30-60 min at 90 volts.

RNA transfer. 20-25 μg of RNA was denatured in RNA loading dye (Sigma) for 10-min at 65^0C followed by incubation on ice for 5-min. Denatured RNA was loaded onto an RNA gel containing 1% agarose, 1X MOPS, 0.1-0.25 μg/ml ethidium bromide and 2% formaldehyde (5.5 ml 36.5% stock in 100 ml solution). The gel was run at 80-85 volts for 2-3 hrs and then photographed using the gel documentation system from Alpha Innotech (Santa Clara, California). The gel was soaked twice in 10X SSC for 15-min to remove the formaldehyde. RNA was transferred from the gel to a positively charged nylon membrane (Roche, Indianapolis) by capillary blotting with 10X SSC for 16-18 hrs, and the transferred RNA was stabilized onto the filter by UV cross-linking (UVP, Upland, California). The RNA-containing membrane was stored at 4^0C.

Preparation of DIG labeled Probe. A PCR based method was used to prepare DIG labeled probes for four target transcripts and an internal control of 18s rRNA. The primers used for PCR amplification and the lengths of probes are listed in **Table 2**. A typical 50 μl labeling reaction consisted of 5 μl HotMaster Taq Buffer (Fisher BioReagents), 2-5 μl of 10 μM forward primer, 2-5μl of 10 μM reverse primer, 5μl DIG-labeled dNTP (Roche, Indianapolis), 5-30 ng plasmid template, 0.50-0.75 μl HotMaster Taq polymerase, and dH_2O for a total volume of 50 μl. PCR conditions were specific for each DIG-labeled probe. The DIG-labeled probe was checked on 1% agarose gel and always ran slower than the control PCR product. The probe was denatured before use by incubating for 5 min at 100^0C and then transferring to ice.

Hybridization. Nylon membranes containing transferred RNA were pre-hybridized in 40 ml pre-warmed DIG Easy Hybridization solution (Roche, Indianapolis) for at least 1-4 hrs at 20-25 rpm in a hybridization oven set at 42^O C (Amerex Instruments). The hybridization solution was replaced by a mix of 40 ml fresh pre-warmed hybridization solution and 25-50μl of denatured DIG-labeled probe, and the membrane was incubated in this mix for 3-16 hrs at 42^O C. The hybridization solution can be store at -20^0C and reused. The reused hybridization solution was heated at 68^0C for 10 minutes before use.

Table 2. Primers and Probes Used for Expression Analysis of Potato Genes

Probe name	Hybridization target	Primer sequences	Probe size (bp)
ASN1	Asn1 transcript	F: GGTTGATGACTGATGTCCCCTTTG R: TAGTTAGCTCCTTATTGTGAGCTC	1115
PPO[1]	Ppo5 transcript	F: ATCTTCCACTCCTAAGCCCTCTCAAC R: CCGCCAAAGAACATCCGAGG	864
PHL	PhL transcript	F: CAATTCCAGATTCATCCATTTCAC R: CATCCCCAGGGTAGAGTATGTAAC	968
R1	R1 transcript	F: ACCAGGGATTCCTAACCTCAAC R: CAGGTATATCACTCTTTGTTAC	852
18s	18s rRNA	F: GCATTTGCCAAGGATGTTTT R: GTACAAAGGGCAGGGACGTA	694

[1]The PPO probe was derived from a cDNA of the *Ppo5* gene of *S. tuberosum*, which is highly expressed in tubers. The probe also hybridized with *Ppo* genes expressed in other tissues. The sequence in pSIM1278 used to silence the *Ppo5* gene consists of the untranslated trailer of a *Ppo* gene from *S. verrucosum*.

Detection. The hybridization solution was removed and replaced by 100 ml washing solution I (2X SSC/0.1% SDS). Nylon membranes were washed twice in washing solution I for 10 min at RT in the hybridization oven at 25-30 rpm. This low stringency buffer was poured off and preheated high stringency washing solution II (0.5X SSC/ 0.1% SDS) was immediately added. The membrane was washed twice in washing solution II at 60^0C for 20 min each at 25-30 rpm. This was followed by a wash for 20 min with washing solution III (0.1X SSC/ 0.1% SDS) at 65^0C and a brief rinse with 2X SSC to remove SDS. The membrane was rinsed with 100 ml 1X DIG Washing Solution (Roche, Indianapolis) in a tray at room temperature for 2 min. and incubated in 1X Blocking solution (Roche, Indianapolis) for 30 min. to 3 hrs on a shaker. The blocked membrane was incubated with DIG antibody solution (1:10,000 dilution of Anti-DIG-alkaline phosphate conjugate with 1X Blocking solution, Roche, Indianapolis) for 30 min on a shaker. The membrane was washed twice (15 min each) with 1X DIG Washing Solution 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 exposed to Z-ray film in the dark. Depending on the experiment multiple exposures were taken from 30 sec to 10 min). The films were developed with Konica SRX-101A X-ray film developer. The x-ray films were scanned to obtain electronic images.

CONCLUSIONS

The targeted four genes (*Asn1, Ppo5, PhL, and R1*) are partially silenced in tubers of most events, except for J78 and G11 which do not contain the cassette for silencing the *PhL/R1* genes (**Table 1**). In addition the *PhL* gene did not appear down-regulated in tubers of the Ranger Russet events, F10 or F37. The *Asn1 and Ppo5* genes are down-regulated in stolons, roots and stems for some of the events. The *Asn1* gene was down regulated in flowers of all events with the exception of the H variety that does not produce flowers. These results indicate that the promoters used for gene silencing are not entirely tissue-specific.

Considering the potato events in this study, expression of the targeted genes would provide evidence that the desired phenotype with low asparagine, reduced black spot bruise, and lowered reducing sugars had been achieved. It could provide information relevant to the plant pest risk assessment, for example, all Innate™ potatoes in the study had reduced or similar expression to the controls. Thus, the altered expression is unlikely to affect weediness or other plant pest characteristics. Also, with these events, the expression studies pertain to endogenous proteins rather than new proteins that could require a safety evaluation, such as those associated with herbicide tolerance or insect resistance. The altered gene expression levels did not affect the agronomic characteristics or tuber composition of the event compared to the conventional untransformed variety.

Supporting data for these conclusions are available in **Appendix 6. Field Performance and Tuber Evaluations** and **Appendix 9. Compositional Analyses**.

Event Ranger Russet F10: The targeted genes are at least partially silenced in tubers, except for *PhL,* and all but *Ppo5* are silenced in stolons. The *Asn1*, *Ppo5, and R1* genes are down-regulated in roots, the *Ppo5* gene in stems, and the *Asn1* gene in leaves and flowers (**Table 1**).

Event Ranger Russet F37: The targeted four genes are at least partially silenced in tubers, except for *PhL,*, and all but *Ppo5* are silenced in stolons. Some of these are also down-regulated in expression, at least partially, in roots (*Asn1*, *Ppo5, and R1*), stems (*Asn1 and Ppo5*), and flowers (*Asn1* and *R1*) (**Table 1**).

Event Russet Burbank E12: The targeted four genes are at least partially silenced in tubers, and all but *Ppo5* are silenced in stolons . Some of these are also down-regulated in expression, at least partially, in roots (*Asn1* and *Ppo5*), stems (*Ppo5*), and flowers (*Asn1*) (**Table 1**).

Event Russet Burbank E24: The targeted four genes are at least partially silenced in tubers and stolons. Some of these are also down-regulated in expression, at least partially, in stems (*Asn1* and *Ppo5*) and flowers (*Asn1*) (**Table 1**).

Event Atlantic J3: The targeted four genes are at least partially silenced in tubers, and *Asn1* and *R1* are silenced in stolons. Some of these are also down-regulated in expression, at least partially, in roots (*Asn1*), stems (*Asn1 and Ppo5*), and flowers (*Asn1*) (**Table 1**).

Event Atlantic J55: The targeted four genes are at least partially silenced in tubers, and all but *Ppo5* are silenced in stolons. Some of these are also down-regulated in expression, at least partially, in roots (*Asn1*), stems (*Ppo5*), and flowers (*Asn1*) (**Table 1**).

Event Atlantic J78: The *Asn1* and *Ppo5* genes are silenced in tubers, the *Asn1* gene is silenced in stolons, the *Ppo5* gene is silenced in stems, and the *Asn1* gene is silenced in flowers (**Table 1**). Silencing was not observed for the *PhL/R1* genes, as expected, since J78 does not contain the cassette for silencing the *PhL/R1* genes.

Event G11: The *Asn1* and *Ppo5* genes are silenced in tubers and stolons; the *R1* gene is partially silenced in leaves and stems; and the *Asn1* gene is silenced also in flowers (**Table 1**). Silencing in tubers and stolons was not observed for the *PhL/R1* genes, as expected since G11 does not contain the cassette for silencing the *PhL/R1* genes. The lower expression of *R1* in leaves and stems was not expected. Such unanticipated reductions in gene expression in non-tuber tissues were not associated with agronomic differences in the field and are, therefore, not expected to have any impact on the safety of G11 plants or their pest potential.

Event H37: The targeted four genes are at least partially silenced in tubers, and *PhL* and *R1* are silenced in stolons. Some of these are also down-regulated in expression, at least partially, in roots (*Ppo5*), stems (*Ppo5*), and *R1* is silenced in leaves (**Table 1**).

Event H50: The targeted four genes are at least partially silenced in tubers and stolons. Some of these are also down-regulated in expression, at least partially, in roots (*Asn1*, *Ppo5,* and *R1*), stems (*Asn1* and *Ppo5*), and *R1* is silenced in leaves (**Table 1**).

REFERENCES

Anand A, Zhou T, Trick HN, Gill BS, Bockus WW, Muthukrishnan S (2003) Greenhouse and field testing of transgenic wheat plants stably expressing genes for thaumatin-like protein, chitinase and glucanase against *Fusarium graminearum*. J Exp Botany, 54: 1101-1111.

Kuipers GJ, Vreem TM, Meyer H, Jacobsen E, Feenstra WJ,, Visser RGF (1992) Field evaluation of antisence RNA mediated inhibition of GBSS gene expression in potato. Euphytica, 59: 83-91.

Mikschofsky H, Heilman E, Schmidtke J, Schmidt K, Meyer U, Leinweber P, Broer I (2011) Greenhouse and field cultivations of antigen-expressing potatoes focusing on the variability in plant constituents and antigen expression. Plant Mol Biol, 76: 131-144.

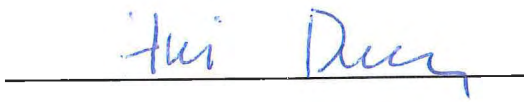
Molnar A, Melnyk CW, Bassett A, Hardcastle RD, Baulcombe DC (2010) Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science, 328: 872-875.

Nakata PA, Anderson JM, Okita TW (1994) Structure and expression of the potato ADP-glucose pyrophosphorylase small subunit. J Biol Chem, 269: 30798-30807.

Visser RGF, Stolte A, Jacobsen E (1991) Expression of chimaeric granule-bound starch synthase – GUS gene in transgenic potato plant. Plant Mol Biol, 17: 691-699.

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.



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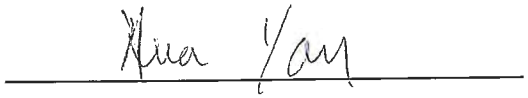

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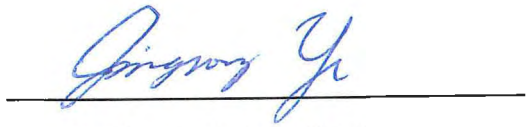

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Appendix 6
Field Performance and Tuber Evaluations

INDEX

INTRODUCTION

Plants and tubers of the events were compared phenotypically to those of untransformed parent varieties in diverse growing locations to determine whether the transformation had affected agronomic performance, ecological interactions, and/or tuber yield and quality. The assessments made it possible to select for events that displayed the intentionally incorporated new traits and maintained all beneficial traits of the conventional varieties (from among a larger number of events). They also made it possible to ensure that the transformations had not resulted in the introduction of unintended effects associated with weediness or pest-like characteristics.

During 2009, 2010, and 2011, plants of the ten events and their untransformed counterparts were grown at multiple locations representing the major production areas for the potato varieties used (**Table 1**).

Table 1. Field Trial Locations

USDA Notification #	Site State	Site County	Material Tested	Trial Design[3]	Rows x Planted Tubers/ Rep	Seed Type	Regional Specifics	Location ID Code
2009 Field trials								
09-049-114n	ID	Canyon	Ranger Russet, Russet Burbank, Atlantic	RCB, 5 reps/line (1 rep/line for Atlantic)	1x20 (1x15 for Atlantic)	Mini-tubers	Typical for Southwest Idaho, an important potato-growing region in a state in the Northwest that produces about 120 million cwt/year, mainly for the French fry industry, Careful management is needed to limit or prevent heat-associated agronomic issues.	Not required[1]
09-049-114n	ID	Bingham	Ranger Russet, Russet Burbank	RCB, 5 reps/line	1x20	Mini-tubers	Typical for Southeast Idaho, an important potato-growing region in a state that produces about 120 million cwt/year, mainly for the French fry industry, with harvests in Fall. The growing season is relatively short because of cooler temperatures.	Not required[1]
09-049-114n	WI	Oneida	G, H	1 rep/line	1x100	Mini-tubers	Typical for Wisconsin, a Midwest state that produces about 20 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall. Large areas are dominated by muck soils.	Not required[1]
08-353-103n	FL	St. Johns	G, H	RCB, 4 reps/line	1x25	Mini-tubers	Typical for Florida that produces almost 8 million cwt/year, mainly for the chip and fresh potato industry, with harvests in Spring.	Not required[1]
08-353-104n	MI	Montcalm	Ranger Russet, Russet Burbank, G, H	RCB, 4 reps/line	1x20	Mini-tubers	Typical for Michigan, a Midwest state that produces about 15 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall. The climate is characterized by mild temperatures and ample rain.	Not required[1]

Table 1 (Continued). Field Trial Locations

USDA Notification #	Site State	Site County	Material Tested	Trial Design[3]	Rows x Planted Tubers/ Rep	Seed Type	Regional Specifics	Location ID Code
2010 Field trials								
10-053-132n	ID	Canyon	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line	3x20	Field seed (G1) (Mini-tubers for Atlantic)	*See above for Canyon county*	UOIPAR2010
10-053-132n	ID	Bingham	Ranger Russet, Russet Burbank, Atlantic, G, H	RCB, 3 reps/line (4 reps/line for G, H; 5 reps/line for Atlantic)	3x20 (1x20 for G, H, Atlantic)	Field seed (G1) (Mini-tubers for Atlantic)	*See above for Bingham county*	UOIABE2010
10-053-132n	WI	Oneida	G, H	RCB, 4 reps/line	1x20	Field seed (G1)	*See above for Oneida county*	RHI2010
10-053-132n	WI	Adams	Atlantic	RCB, 5 reps/line	1x20	Mini-tubers	Typical for Wisconsin, a Midwest state that produces about 20 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall.	WI2010
10-076-013n	WI	Vilas	G, H	1 rep	Seed bulkup[4]	Mini-tuber/ G1	Typical for Wisconsin, a Midwest state that produces about 20 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall.	VILAS2010
10-053-132n	MI	Montcalm	Atlantic, G, H	RCB, 3 reps/line (4 reps/line for G, H)	3x20 (1x20 for G, H)	Field seed (G1) (Mini-tubers for Atlantic)	*See above for Montcalm county*	MSUMONT2010
10-053-132n	MI	Missaukee	G, H	RCB, 4 reps/line	1x20	Field seed (G1)	Typical for Michigan, a Midwest state that produces about 15 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall.	MSULAKE2010
10-053-132n	NE	Cherry	Atlantic	RCB, 5 reps/line	1x20	Field seed (G1) (Mini-tubers for Atlantic)	Typical for Nebraska, a Midwest state that produces about 9 million cwt/year, mainly for the seed industry, with harvests in Fall.	CSS2010
10-053-132n	ND	Grand Forks[2]	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line (5 reps/line for Atlantic)	3x20 (1x20 for Atlantic)	Field seed (G1) (Mini-tubers for Atlantic)	Typical for North Dakota, a Midwest state that produces about 20 million cwt/year, mainly for the French fry industry, with harvests in Fall. The Red River Valley is the 3rd largest potato growing region in the US.	NDSU2010
10-053-132n	WA	Adams	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line (5 reps/line for Atlantic)	3x20 (1x20 for Atlantic)	Field seed (G1) (Mini-tubers for Atlantic)	Typical for Washington, a state in the Northwest that produces about 85 million cwt/year, mainly for the French fry industry, with harvests in Fall. Ideal growing conditions give rise to very high yields per acre.	WSU2010
09-336-103n	FL	St. Johns	G, H	RCB, 4 reps/line	1x25	Field seed (G1)	*See above for St. Johns county*	UFL2010

Table 1 (Continued). Field Trial Locations

USDA Notification #	Site State	Site County	Material Tested	Trial Design[3]	Rows x Planted Tubers/ Rep	Seed Type	Regional Specifics	Location ID Code
2011 Field trials								
11-063-103n	ID	Canyon	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line	3x20	Field seed (G2) (G1 for Atlantic, Mini-tubers for F37)	*See above for Canyon county*	UOIPAR2011
11-063-103n	ID	Bingham	Ranger Russet, Russet Burbank, Atlantic,	RCB, 3 reps/line	3x20	Field seed (G2) (G1 for Atlantic, Mini-tubers for F37)	*See above for Bingham county*	UOIABE2011
11-063-103n	WI	Adams	Atlantic	RCB, 3 reps/line	3x20	Field seed (G1)	*See above for Adams county, Wisconsin*	WI2011
11-063-103n	MI	Montcalm	Atlantic	RCB, 3 reps/line	3x20	Field seed (G1)	*See above for Montcalm county*	MSUMONT2011
11-063-103n	ND	Grand Forks	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line	3x20	Field seed (G2) (G1 for Atlantic, Mini-tubers for F37)	*See above for Grand Forks county*	NDSU2011
11-063-103n	WA	Adams	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line	3x20	Field seed (G2) (G1 for Atlantic, Mini-tubers for F37)	*See above for Adams county, Washington*	WSU2011
10-326-103n	FL	St. Johns	G, H, Atlantic	RCB, 4 reps/line	1x20	Field seed (G1)	*See above for St. Johns county*	UFL2011
11-063-103n	WI	Oneida	G, H	RCB, 4 reps/line	1x20	Field seed (G1)	*See above for Oneida County*	RHI2011
11-063-103n	IN	Pulaski	Atlantic	RCB, 3 reps/line	1x20	Field seed (G1)	Typical for Indiana, a Midwest state that produces about 2 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall.	Indiana2011
11-094-106n	WI	Oneida	G,H	RCB, 4 reps/ line	1x13	Field seed (G1	*See above for Oneida County*	ONEIDA2011

[1]USDA/APHIS did not require a unique location identification code in 2009.
[2]Because parts of this field were flooded extensively, Ranger Russet and Russet Burbank tubers were evaluated only biochemically.
[3]Randomized Complete Block Designs contained the same number of blocks as the number of reps in the table.
[4]This was a seed bulkup site, no data were collected.

The agronomic practices and pest control measures employed were location-specific and typical for potato cultivation. They were recommended by both regional potato extension specialists and corporate agronomists and related to all aspects of soil preparation, fertilizer application, irrigation, and pesticide-based control methods. Events and untransformed varieties received identical inputs and treatments within each site. The trial sites selected for the agronomic evaluations were different eco-agricultural zones, and represented the main production areas for potatoes that are destined for fry and chip production in the USA.

Field evaluations were carried out by agronomists and scientists with extensive experience in growing and analyzing potatoes. The traits evaluated are listed in **Table 2** and are used by plant breeders in Plant

Variety Protection (PVP) filings. These traits might help identify plants displaying characteristics that are typical of pests or noxious weeds and, therefore, of interest to the USDA-APHIS.

Table 2. Characteristics Evaluated

General characteristic	Characteristic measured	Evaluation timing[1]	Data description	Scale[2]
Early Emergence	Emergence	30 days after planting	% emergence	Percent based on number of seed pieces planted
Final Emergence	Emergence	Early season	% emergence	Percent based on number of seed pieces planted
Insect Stressors	Population	Early, Mid, & Late season	Visual estimate of relative population	1 to 5 point scale; 1=high population, 3=average population, 5=low population
Disease Stressors	Symptoms	Early, Mid, & Late season	Visual estimate of relative pressure	1 to 5 point scale; 1=high pressure, 3=average pressure, 5=low pressure
Abiotic Stressors	Symptoms	Early, Mid, & Late season	Visual estimate of relative symptom expression	1 to 5 point scale; 1=high symptom expression, 3=average symptom expression, 5=low symptom expression
Stems Per Plant	Stems per Plant	Early season	Count of stems per plant	Number of stems per plant
Incidental Stressors	Diseases	Mid & Late season	Observations	Presence/absence
	Insects	Midseason	Observations	Presence/absence
Above ground characteristics	Plant Vigor	Midseason	Visual estimate of relative vigor	1 to 5 point scale; 3=control/normal, 1= less or worse than control/normal, 5 = greater or better than control/normal
	Foliage Color	Midseason	Visual estimate of relative foliage color	1 to 5 point scale; 3=control/normal, 1= less or worse than control/normal, 5 = greater or better than control/normal
	Leaflet Size	Midseason	Visual estimate of relative leaflet size	1 to 5 point scale; 3=control/normal, 1= less or worse than control/normal, 5 = greater or better than control/normal
	Leaflet Curl	Midseason	Visual estimate of relative leaflet curl	1 to 5 point scale; 3=control/normal, 1= less or worse than control/normal, 5 = greater or better than control/normal
	Flower Color	Midseason	Observation of flower color	Flower color recorded as purple, white, or mixed per plot
	Senescence	Late season	Visual estimate of % necrotic foliage at maturity	% of necrotic vine per plot
	Vine Size	Late season	Visual estimate of relative vine size	1 to 5 point scale; 3=control/normal, 1= less or worse than control/normal, 5 = greater or better than control/normal
	Vine maturity	Late season	Visual estimate of relative vine maturity	1 to 5 point scale; 3=control/normal, 1= less or worse than control/normal, 5 = greater or better than control/normal

[1]Early season observations were made at approximately 45 days after emergence. Midseason observations were made during the early bloom stage. Late season notes were taken during the crop senescence stage.

[2] While it was expected when using the 1 to 5 scale that all controls would have mean values of 3, however, some observers treated the scale as if 3 were normal, rather than a comparison to the control.

Table 2 (Continued). Characteristics Evaluated

General characteristic	Characteristic measured	Evaluation timing[1]	Data description	Scale
Yield	Total yield	After harvest	Plot weight	Lbs./row of 20 seed pieces
	Tuber grading	After harvest	Tubers categorized as 4-6 oz., 6-10 oz., 10-14 oz., >14 oz., U.S. #1, Grade B, Grade A, Oversize, or Pick Out, as appropriate	% of tubers by weight in each category
	Specific gravity	After Harvest	Tuber sample weight in air/(weight in air - weight in water)	Numeric specific gravity value
	High Sugar	After Harvest	Color rating of test fried strips based on USDA Color Chart for French Fried Potatoes	% of tubers with >1/2 of the fried strip has a number 3 or darker color
	Sugar ends	After Harvest	Color rating of the end of test fried strips based on USDA Color Chart for French Fried Potatoes	% of tubers with an end ¼" long or longer on the darkest end of the strip, testing number 3 or darker color
	Total Internal Defects	After Harvest	Sum of internal defects such as hollow heart, vascular discoloration, brown center, etc.	% of internal defects

[1]Early season observations were made at approximately 45 days after emergence. Midseason observations were made during the early bloom stage. Late season notes were taken during the crop senescence stage.

BQMS Quality Management Systems: All agronomic trials were conducted according to USDA Biotechnology Quality Management System (BQMS) standards and were USDA-APHIS compliant. Compliance to USDA requirements described in CFR 7 part 340 was assured by:

(1) Providing details on the introduced DNA in the submitted Design Protocol
(2) Confirming that plants and tubers are free of *Agrobacterium*
(3) Monitoring eight critical control points (CCPs) in the process of transporting, evaluating, harvesting, storing, and eliminating potatoes that are no longer needed. The associated documents were developed in collaboration with APHIS/BQMS and include: (1) Record of Transport indicating the USDA movement permit number, (2) Record of Storage and Inventory Change, (3) Record of Planting, (4) Record of Spatial Isolation, (5) Record of Harvest, (6) Record of Processing, (7) Record of Post-Harvest Monitoring, which commits to monitoring trials for the establishment of volunteers during the two years following an experiment, and (8) Record of Corrective Action.

Both internal and external audits were undertaken to inspect all records and ensure that regulated plants or tubers were not lost or mixed with other plants or tubers during transport, planting, and storage.

RESULTS and DISCUSSION

Interpretation of the Results

For each comparison between a test line and the appropriate conventional control, the following logic was employed to assess the results. First, when p-values were available, statistical significance was determined for each comparison. If the p-value indicated no statistical significance, it was unlikely that there was a difference that would be biologically meaningful or indicative of increased pest potential. Next, if the p-value indicated statistical significance or if a p-value was not present, the mean value of the test line was compared to the combined control range, which represents the minimum and maximum mean values of all conventional lines included in the experiments. If the value for the test line was within the combined control range, it was concluded that the test line exhibited characteristics within the natural variation of potatoes and that the difference was unlikely to be biologically meaningful or indicative of increased pest potential. If the value of the test line was outside the combined control range, further consideration was given to the difference in the context of agronomic impact and increased pest potential.

The combined control range for the agronomic characteristics used a total of 319 mean values from conventional Ranger, Burbank, Atlantic, G, and H varieties grown as controls in the field trials. For the yield and grading data for the Burbank and Ranger lines, the combined control range used a total of 170 mean values of the Burbank and Ranger conventional controls. For the yield and grading data for the Atlantic, G, and H lines, a total of 160 mean values of Atlantic, G, and H conventional controls were used to determine the combined control range.

Field Performance and Tuber Assessment for Ranger Russet Events F10 and F37

Evaluations of phenotypic characteristics and ecological interactions (stressors) of the Ranger Russet F10 and F37 events and controls grown in 2009, 2010, and 2011 are shown in **Tables 3-7**. Results were analyzed by statistical methods where possible. Overall the data suggest that there are no major differences between the Ranger Russet control and the F10 and F37 Ranger Russet events.

The agronomic characteristics for F10 and the Ranger control are shown in **Table 3.** No statistically significant differences were detected between F10 and the control for any agronomic characteristic. Foliage color and vine maturity rating data were not able to be statistically compared; however, the observed values of F10 for foliage color and vine maturity rating were within the combined control range.

The agronomic characteristics for F37 and the Ranger control are shown in **Table 3.** There were no differences detected between F37 and the control for early emergence, final emergence, leaflet curl, or senescence. Foliage color and vine maturity rating data were not able to be statistically compared; however, the observed values of F37 for both characteristics were within the combined control range. Three significant differences were detected between F37 and the control for stems per plant (1.5 vs. 2.5), plant vigor (3.4 vs. 3.1), and leaflet size (3.3 vs. 3.0). The value of F37 for plant vigor was within the combined control range. The values of F37 for stems per plant and leaflet size were outside of the combined control range (1.5 vs. 1.6-5.0 and 3.3 vs. 2.0-3.0, respectively), but these differences were small and would not contribute to increased weediness or pest potential.

The flower colors of F10, F37, and the Ranger control are shown in **Table 4**. All flowers observed were purple or mixed, as expected.

The yield and grading characteristics of F10 and the Ranger control are shown in **Table 5**. There were no statistically significant differences detected for the 6-10 oz. tuber size category, >14 oz. category, specific gravity, high sugar, or sugar ends. Statistical analysis was not possible for total internal defects but the values for F10 were within the combined control range. Three differences were detected between F10 and the control for total yield (53.7 vs. 60.0, respectively), the 4-6 oz. category (23.9 vs. 18.4), and the 10-14 oz. category (13.9 vs. 19.3). All values for F10 were within the combined control range for these characteristics.

The yield and grading characteristics of F37 and the Ranger control are shown in **Table 5**. There were no differences detected between F37 and the control for five characteristics: the 4-6oz. category, the 10-14 oz. category, the >14 oz. category, specific gravity, and sugar ends. Total internal defects could not be statistically analyzed and the mean value of F37 for total internal defects was outside the combined control range (3.4 vs. 0.0-0.7); however, this difference would not contribute to increased weediness or pest potential. Significant differences were detected between F37 and the control for total yield (45.4 vs. 60.0), the 6-10 oz. category (29.4 vs. 35.5), and high sugar (14.7 vs. 0.3). All values of F37 for these three characteristics were within the combined control range.

The insect, disease, and abiotic stressor evaluations for F10 and the Ranger control are shown in **Table 6**. No differences were noted between F10 and the control during 42 individual observations of four insect stressors. For the disease stressors, 46 of 48 observations lacked observed differences between F10 and the control. No differences between F10 and the control were observed during 36 observations of the five abiotic stressors. An observation is described as looking for an insect, disease, or abiotic stressor during a point in time. Even if no stressors are present, 0's were recorded because the stressors were looked for and therefore comparisons can be made beteween the events and the conventional controls. The two observed differences between F10 and the control are detailed in the footnotes of **Table 6**. The lack of differences between F10 and the control for stressor observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

The insect, disease, and abiotic stressor evaluations for F37 and the Ranger control are shown in **Table 6**. No differences were noted between F37 and the control during 29 out of 30 individual observations of four insect stressors. For the disease stressors, 33 of 36 observations lacked observed differences between F37 and the control. No differences between F37 and the control were observed during 21 observations of the five abiotic stressors. The four observed differences between F37 and the control are detailed in the footnotes of **Table 6**. The lack of differences between F37 and the control for stressor observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

Notes were also taken on incidental stressors for F10, F37, and the Ranger control and are presented in **Table 7**. No incidental insect stressors were found for F10, F37, or the control during 27 observations. Out of 47 observations, incidental *Verticillium* was noted five times for F10, three times for F37, and five times for the control. No incidental virus was found out of 47 observations for F10, F37, or the control. Other incidental stressors were identified three times each for F10, F37, and the control out of 44 observations.

It can be concluded that there are no major differences in agronomic characteristics, flower color, yield and grading, and ecological interactions between the untransformed Ranger Russet variety and events F10 and F37 (**Tables 3, 4, 5, 6, and 7**). Therefore, based on our multi-year data, the Ranger Russet events F10 and F37 pose no significant risk of persistence in the environment as a result of weediness or increased plant pest potential.

Table 3. Ranger Russet 2009-2011 Field Trials – Agronomic Characteristics

Entry	Statistic	Characteristic (see **Table 2.** for descriptions)									
		Early Emergence	Final Emergence	Stems Per Plant[1]	Plant Vigor	Foliage Color	Leaflet Size	Leaflet Curl	Senescence	Vine Size[2]	Vine Maturity Rating
F10	Mean	87.9%	94.3%	2.5	3.3	3.2	3.0	2.3	30.6	.	2.7
	SD	13.0%	6.9%	0.35	0.60	0.47	0.08	0.93	14.02	.	0.71
	90% CI	81.2-94.7%	92.4-96.2%	2.34-2.71	3.13-3.46	3.05-3.31	2.99-3.03	2.08-2.58	21.87-39.25	.	2.40-2.91
	p-Value	1.0000	0.1700	0.8433	0.0659	NA	0.8171	0.1401	0.6270	.	NA
F37	Mean	82.5%	93.8%	1.5	3.4	3.3	3.3	2.1	27.2	.	3.3
	SD	14.5%	6.5%	0.19	0.79	0.46	0.45	1.01	20.33	.	0.92
	90% CI	75.0-90.0%	91.9-95.6%	1.41-1.61	3.11-3.59	3.17-3.46	3.13-3.41	1.75-2.38	14.62-39.82	.	3.00-3.66
	p-Value	0.4898	0.0687	0.0001	0.0237	NA	0.0001	0.4108	0.8075	.	NA
Ranger Ctrl	Mean	87.9%	96.0%	2.5	3.1	3.0	3.0	2.2	28.3	.	3.0
	SD	24.2%	6.2%	0.49	0.46	0.32	0.00	0.99	13.46	.	0.10
	90% CI	75.4-100%	94.2-97.7%	2.24-2.76	2.93-3.17	2.86-3.04	3.00-3.00	1.96-2.50	19.99-36.68	.	2.98-3.06
ComC[3]	Range	7.0-100.0	79.0-100.0	1.6-5.0	2.3-4.3	2.3-4.0	2.0-3.0	1.0-3.0	8.7-91.7	.	2.3-4.5

[1]Stems per plant data from 2011 only.
[2]Vine size data not collected.
[3]ComC = combined control range. The range of mean values of conventional Ranger, Burbank, Atlantic, G, and H controls.
NA = Statistical comparison not possible.

Table 4. Ranger Russet 2011 Field Trials – Flower Color Characteristics

Entry	Number of Plots	
	Purple or Mixed Flowers	White Flowers
F10	12	0
F37	6	0
Ranger Ctrl	12	0

Table 5. Ranger Russet 2009-2011 Field Trials – Yield and Grading Characteristics

Entry	Statistic	Characteristic								
		Total Yield	4-6 oz	6-10 oz	10-14 oz	>14 oz	Specific Gravity[1]	High Sugar	Sugar Ends	Total Internal Defects
F10	Mean	53.7	23.9	35.6	13.9	9.9	1.095	1.3	3.0	0.0
	SD	20.8	9.6	8.8	8.4	10.4	0.008	3.1	4.7	0.0
	90% CI	47.1-60.3	19.8-27.9	31.9-39.4	10.4-17.5	5.5-14.3	1.092-1.099	0.0-2.6	1.0-5.0	0.0-0.0
	p-Value	0.0083	0.0108	0.7564	0.0323	0.0638	0.4808	0.8457	0.7387	NA
F37	Mean	45.4	18.3	29.4	16.0	13.3	1.096	14.7	4.0	3.4
	SD	24.7	7.2	7.9	9.4	12.0	0.009	27.8	7.1	5.6
	90% CI	37.7-53.1	15.2-21.3	26.1-32.8	12.0-19.9	8.2-18.4	1.092-1.100	2.9-26.4	1.0-7.0	0.4-6.3
	p-Value	0.0001	0.7573	0.0054	0.2431	0.5446	0.3735	0.0127	0.4323	NA
Ranger Ctrl	Mean	60.0	18.4	35.5	19.3	15.2	1.096	0.3	2.5	0.0
	SD	21.1	10.0	9.5	8.5	16.9	0.008	0.8	4.1	0.0
	90% CI	53.3-66.6	14.3-22.5	31.6-39.4	15.8-22.8	8.3-22.2	1.093-1.100	0.0-0.7	0.8-4.2	0.0-0.0
ComC[2]	Range	26.0-107.7	2.3-32.2	8.8-51.6	6.7-31.7	0.0-59.3	1.071-1.112	0.0-36.1	0.0-70.3	0.0-0.7

[1]Specific gravity data from 2011 only.

[2]ComC = combined control range. The range of mean values of conventional Ranger and Burbank controls.

NA = Statistical comparison not possible.

Table 6. Ranger Russet 2010-2011 Field Trials – Stressor Observations

Stressor	F10		F37	
	Total # Obs.	# Obs. Without Observed Differences*	Total # Obs.	# Obs. Without Observed Differences*
Insect				
Aphids	18	18	12	12
Colorado Potato Beetle	18	18	12	12
Looper	3	3	3	3
Seed Corn Maggot	3	3	3	2[1]
Totals	42	42	30	29
Disease				
Black Leg	3	3	3	3
Botrytis	12	12	6	5[2]
Early Blight	15	14[3]	9	7[4]
Rhizoctonia	3	3	3	3
Verticillium	3	2[5]	3	3
Virus	3	3	3	3
White Mold	9	9	9	9
Totals	48	46	36	33
Abiotic				
Frost	3	3	3	3
Hail	12	12	9	9
Heat	3	3	3	3
Herbicide	15	15	6	6
Wind	3	3	0	0
Totals	36	36	21	21

*Stressor evaluations were intended to be categorical and were not statistically analyzed. The range of ratings for each event and its control were compared for each observation and an observed difference occurred when the range of the event was outside the range of the control.

[1]During one observation F37 ranged 3-3 and control ranged 5-5 (Adams Co., WA 2011).

[2]During one observation F37 ranged 4-4 and control ranged 0-3 (Canyon Co., ID 2011).

[3]During one observation F10 ranged 0-5 and control ranged 4-5 (Grand Forks Co., ND 2011).

[4]During two observations F37 ranged 4-5 and 3-5 and control ranged 0-4 and 3-4.5, respectively (Bingham Co., ID 2011 and Canyon Co., ID 2011).

[5]During one observation F10 ranged 3-4.5 and control ranged 4-5 (Bingham Co., ID 2011).

Table 7. Ranger Russet 2009-2011 Field Trials – Incidental Stressor Observations[1]

Entry	Incidental Insect		Incidental *Verticillium*		Incidental Virus		Incidental Other	
	Count of No	Count of Yes	Count of No	Count of Yes	Count of No	Count of Yes	Count of No	Count of Yes
F10	27	0	42	5	47	0	41	3
F37	27	0	44	3	47	0	41	3
Ranger Ctrl	27	0	42	5	47	0	41	3

[1]Specific stressors, except *Verticillium*, were not identified during the incidental stressor observations and only presence/absence data were collected. Counts are the number of observations where incidental stressors were found (yes) or looked for but not found (no).

Field Performance and Tuber Assessment for Russet Burbank Events E12 and E24

Evaluations of phenotypic characteristics and ecological interactions (stressors) of the Russet Burbank E12 and E24 events and controls grown in 2009, 2010, and 2011 are shown in **Tables 8-12**. Results were analyzed by statistical methods where possible. Overall the data suggest that there are no major differences between the Russet Burbank control and the E12 and E24 Russet Burbank events.

The agronomic characteristics for E12 and the Burbank control are shown in **Table 8**. No statistically significant differences were detected between E12 and the control for seven of the eight agronomic characteristics which were statistically analyzed. Vine maturity rating data were not able to be statistically compared but the observed value of E12 was within the combined control range. A statistically significant difference was detected between E12 and the control for plant vigor (3.4 vs. 3.1); however, the value of E12 was within the combined control range.

The agronomic characteristics for E24 and the Burbank control are shown in **Table 8**. There were no differences detected between E24 and the control for early emergence, final emergence, foliage color, or senescence. Vine maturity rating data were not able to be statistically compared but the observed value of E24 was within the combined control range. Four significant differences were detected between E24 and the control for stems per plant (3.1 vs. 2.7), plant vigor (3.3 vs. 3.1), leaflet size (3.1 vs. 2.9), and leaflet curl (2.4 vs. 2.2); however, the E24 values for all the characteristics for which differences were detected, were within the combined control range except for leaflet size, where the E24 value was just outside the combined control range (3.1 vs. 2.0-3.0). This difference in leaflet size is small and would not contribute to increased weediness or pest potential.

The flower colors of E12, E24, and the Burbank control are shown in **Table 9**. Both purple/mixed flowers and white flowers were observed in different plots for each entry.

The yield and grading characteristics of E12 and the Burbank control are shown in **Table 10**. There were no statistically significant differences detected for total yield, specific gravity, high sugar, or sugar ends. Statistical analysis was not possible for total internal defects but the values for E12 were within the combined control range. Four differences were detected between E12 and the control for the 4-6 oz. category (25.3 vs. 21.5), the 6-10 oz. category (33.1 vs. 30.3), the 10-14 oz. category (12.6 vs. 16.2), and the >14 oz. category (8.9 vs. 13.1). All values for E12 for these differences were within the combined control range.

The yield and grading characteristics of E24 and the Burbank control are shown in **Table 10**. There were no differences detected between E24 and the control for the 6-10 oz. category, specific gravity, high sugar, and sugar ends. Total internal defects could not be statistically analyzed, but the mean of E24 was within the combined control range. Significant differences were detected between E24 and the control for total yield (52.7 vs. 56.8), the 4-6 oz. category (27.3 vs. 21.5), the 10-14 oz. category (10.7 vs. 16.2), and the >14 oz. category (5.7 vs. 13.1). All values of E24 for these three characteristics were within the combined control range.

The insect, disease, and abiotic stressor evaluations for E12 and the Burbank control are shown in **Table 11**. No differences were noted between E12 and the control during 41 of 42 individual observations of four insect stressors. For the seven disease stressors, 47 of 48 observations lacked observed differences between E12 and the control. No differences between E12 and the control were observed during any of 34 total observations of the five abiotic stressors. The two observed differences between E12 and the

control are detailed in the footnotes of **Table 11**. The lack of differences between E12 and the control for stressor observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

The insect, disease, and abiotic stressor evaluations for E24 and the Burbank control are shown in **Table 11**. No differences were noted between E24 and the control during 41 out of 42 individual observations of four insect stressors. For the seven disease stressors, 46 of 48 observations lacked observed differences between E24 and the control. No differences between E24 and the control were observed during 34 observations of the five abiotic stressors. The three observed differences between E24 and the control are detailed in the footnotes of **Table 11**. The lack of differences between E24 and the control for stressor observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

Notes were also taken on incidental stressors for E12, E24, and the Burbank control and are presented in **Table 12**. No incidental insect stressors were recorded for E12, E24, or the control during 27 observations. Out of 47 observations, incidental *Verticillium* was noted eight times for E12, seven times for E24, and eight times for the control. No incidental virus was found out of 47 observations for E12, E24, or the control. Other incidental stressors were identified three times each for E12, E24, and the control out of 44 observations.

It can be concluded that there are no major differences in agronomic characteristics, flower color, yield and grading, and ecological interactions between the untransformed Russet Burbank variety and events E12 and E24 (**Tables 8, 9, 10, 11, and 12**). Therefore, based on our multi-year data, the Russet Burbank events E12 and E24 pose no significant risk of persistence in the environment as a result of weediness or increased plant pest potential.

Table 8. Russet Burbank 2009-2011 Field Trials – Agronomic Characteristics

Entry	Statistic	Characteristic (see **Table 2.** for descriptions)									
		Early Emergence	Final Emergence	Stems Per Plant[1]	Plant Vigor	Foliage Color	Leaflet Size	Leaflet Curl	Senescence	Vine Size	Vine Maturity Rating[2]
E12	Mean	80.7%	93.7%	2.8	3.4	3.2	3.0	2.3	49.4	.	3.7
	SD	22.7%	8.8%	0.61	0.60	0.38	0.24	0.95	36.27	.	0.90
	90% CI	70.0-91.5%	91.1-96.2%	2.52-3.16	3.22-3.55	3.05-3.26	2.95-3.08	2.05-2.56	26.96-71.92	.	3.33-3.97
	p-Value	0.9367	0.6269	0.2200	0.0096	0.1812	0.0815	0.2051	0.1013	.	NA
E24	Mean	75.0%	93.2%	3.1	3.3	3.2	3.1	2.4	57.8	.	3.7
	SD	26.1%	6.5%	0.68	0.68	0.45	0.22	0.82	39.93	.	0.69
	90% CI	62.7-87.3%	91.3-95.1%	2.77-3.48	3.15-3.52	3.05-3.29	2.99-3.11	2.19-2.63	33.03-82.53	.	3.47-3.96
	p-Value	0.2401	0.4373	0.0032	0.0298	0.1196	0.0138	0.0038	0.1477	.	NA
Burbank Ctrl	Mean	80.4%	94.4%	2.7	3.1	3.1	2.9	2.2	53.9	.	3.1
	SD	21.5%	7.9%	0.50	0.35	0.27	0.27	0.99	36.04	.	0.31
	90% CI	70.2-90.5%	92.1-96.7%	2.41-2.93	2.98-3.17	3.00-3.15	2.85-3.00	1.96-2.50	31.55-76.23	.	2.95-3.18
ComC[2]	Range	7.0-100.0	79.0-100.0	1.6-5.0	2.3-4.3	2.3-4.0	2.0-3.0	1.0-3.0	8.7-91.7	.	2.3-4.5

[1]Stems per plant data from 2011 only.

[2]ComC = combined control range. The range of mean values of conventional Ranger, Burbank, Atlantic, G, and H controls.

NA = Statistical comparison not possible.

Table 9. Russet Burbank 2011 Field Trials – Flower Color Characteristics

Entry	Number of Plots	
	Purple or Mixed Flowers	White Flowers
E12	3	5
E24	3	5
Burbank Ctrl	3	8

Table 10. Russet Burbank 2009-2011 Field Trials – Yield and Grading Characteristics

Entry	Statistic	Characteristic								
		Total Yield	4-6 oz	6-10 oz	10-14 oz	>14 oz	Specific Gravity[1]	High Sugar	Sugar Ends	Total Internal Defects
E12	Mean	53.2	25.3	33.1	12.6	8.9	1.082	12.3	18.0	0.3
	SD	21.8	9.4	7.2	6.6	8.9	0.008	27.7	15.4	0.8
	90% CI	46.3-60.0	21.4-29.1	30.1-36.1	9.9-15.3	5.3-12.6	1.079-1.085	0.9-23.6	11.6-24.3	0.0-0.7
	p-Value	0.2079	0.0061	0.0434	0.0431	.0077	0.1048	0.4455	0.3789	NA
E24	Mean	52.7	27.3	30.1	10.7	5.7	1.083	5.5	19.1	0.5
	SD	22.8	7.7	7.9	5.5	9.3	0.008	9.7	20.0	1.0
	90% CI	45.6-59.8	24.0-30.7	26.6-33.5	8.3-13.1	1.7-9.8	1.079-1.086	1.3-9.8	10.3-27.8	0.0-1.0
	p-Value	0.0061	0.0011	0.8441	0.0115	0.0002	0.2915	0.6629	0.8068	NA
Burbank Ctrl	Mean	56.8	21.5	30.3	16.2	13.1	1.084	8.2	21.4	0.3
	SD	20.9	8.4	8.0	7.3	9.9	0.007	13.9	21.4	0.7
	90% CI	50.5-63.2	18.1-25.0	27.0-33.6	13.2-19.1	9.0-17.2	1.081-1.087	2.4-13.9	12.6-30.2	0.0-0.7
ComC[2]	Range	26.0-107.7	2.3-32.2	8.8-51.6	6.7-31.7	0.0-59.3	1.071-1.112	0.0-36.1	0.0-70.3	0.0-0.7

[1]Specific gravity data from 2011 only.

[2]ComC = combined control range. The range of mean values of conventional Ranger and Burbank controls.

NA = Statistical comparison not possible.

Table 11. Russet Burbank 2009 and 2011 Field Trials – Stressor Observations

Stressor	E12		E24	
	Total # Obs.	# Obs. Without Observed Differences*	Total # Obs.	# Obs. Without Observed Differences*
Insect				
Aphids	18	18	18	18
Colorado Potato Beetle	18	18	18	18
Looper	3	3	3	3
Seed Corn Maggot	3	2[1]	3	2[2]
Totals	42	41	42	41
Disease				
Black Leg	3	3	3	3
Botrytis	12	12	12	12
Early Blight	15	14[3]	15	14[4]
Rhizoctonia	3	3	3	3
Verticillium	3	3	3	3
Virus	3	3	3	3
White Mold	9	9	9	8[5]
Totals	48	47	48	46
Abiotic				
Frost	3	3	3	3
Hail	10	10	10	10
Heat	3	3	3	3
Herbicide	15	15	15	15
Wind	3	3	3	3
Totals	34	34	34	34

*Stressor evaluations were intended to be categorical and were not statistically analyzed. The range of ratings for each event and its control were compared for each observation and an observed difference occurred when the range of the event was outside the range of the control.

[1]During one observation E12 ranged 4-5 and control ranged 4-4 (Adams Co., WA 2011).

[2]During one observation E24 ranged 4-5 and control ranged 4-4 (Adams Co., WA 2011).

[3]During one observation E12 ranged 3-5 and control ranged 3-4.5 (Bingham Co., ID 2011).

[4]During one observation E24 ranged 3-5 and control ranged 3-4.5 (Bingham Co., ID 2011).

[5]During one observation E24 ranged 3-5 and control ranged 4-5 (Bingham Co., ID 2011).

Table 12. Russet Burbank 2009-2011 Field Trials – Incidental Stressor Observations[1]

Entry	Incidental Insect		Incidental *Verticillium*		Incidental Virus		Incidental Other	
	Count of No	Count of Yes	Count of No	Count of Yes	Count of No	Count of Yes	Count of No	Count of Yes
E12	27	0	39	8	47	0	41	3
E24	27	0	40	7	47	0	41	3
Burbank Ctrl	27	0	39	8	47	0	41	3

[1]Specific stressors, except *Verticillium*, were not identified during the incidental stressor observations and only presence/absence data were collected. Counts are the number of observations where incidental stressors were found (yes) or looked for but not found (no).

Field Performance and Tuber Assessment for Atlantic Events J3, J55 and J78

Evaluations of phenotypic characteristics and ecological interactions (stressors) of J3, J55, and J78 events and controls grown in 2010 and 2011 are shown in **Tables 13-17**. Results were analyzed by statistical methods where possible. Overall, the data suggest that there are no major differences between the Atlantic control and the J3, J55, and J78 events.

The agronomic characteristics for J3 and the Atlantic control are shown in **Table 13**. No statistically significant differences were detected between J3 and the control for five of the seven agronomic characteristics which were statistically analyzed. Leaflet curl and vine maturity rating data were not able to be statistically compared; however, the mean values of J3 for these characteristics were within the combined control range. Statistically significant differences were detected between J3 and the control for stems per plant (3.4 vs. 3.1) and senescence (52.4 vs. 47.4); however, the values for J3 were within the combined control range in both cases. Although not significantly different from the control, the value for leaflet size was just outside the combined control range (3.1 vs. 2.0-3.0); however, this small difference would not contribute to increased weediness or pest potential.

The agronomic characteristics for J55 and the Atlantic control are shown in **Table 13**. There were no differences detected between J55 and the control for five of the seven agronomic characteristics which were statistically analyzed. Leaflet curl and vine maturity rating data were not able to be statistically compared; however, the mean values of J55 for these characteristics were within the combined control range. Two significant differences were detected between J55 and the control for early emergence (68.6 vs. 74.3, respectively) and senescence (52.4 vs. 47.4). The values of J55 for both characteristics where differences were detected were within the combined control range.

The agronomic characteristics for J78 and the Atlantic control are shown in **Table 13**. There were no differences detected between J78 and the control for early emergence, final emergence, plant vigor, or foliage color. Leaflet size, leaflet curl, and vine maturity rating data were not able to be statistically compared; however, the mean values of J78 were within the combined control range. Two significant differences were detected between J78 and the control for stems per plant (3.4 vs. 3.1) and senescence (51.9 vs. 47.4). The values of J78 for these characteristics were within the combined control range.

The flower colors of J3, J55, J78, and the Atlantic control are shown in **Table 14**. All lines at all locations in all years had purple or mixed flower color, as expected.

The yield and grading characteristics of J3 and the Atlantic control are shown in **Table 15**. There were no statistically significant differences detected for total yield, % grade A, % pick outs (tubers discarded due to quality issues like rot and mold), or total internal defects. Statistical analysis was not possible for % grade B, but the mean of J3 was within the combined control range. Three statistically significant differences were detected between J3 and the control for % U.S. #1 (84.7 vs. 87.1), % oversize (5.4 vs. 8.3), and specific gravity (1.094 vs. 1.092). All values for J3 for these differences were within the combined control range.

The yield and grading characteristics of J55 and the Atlantic control are shown in **Table 15**. There were no differences detected between J55 and the control for any of the seven analyzed yield and grading characteristics. Percent grade B could not be statistically analyzed, but the mean of J55 was within the combined control range.

The yield and grading characteristics of J78 and the Atlantic control are shown in **Table 15**. There were no differences detected between J78 and the control for any of the seven analyzed yield and grading characteristics. Percent grade B could not be statistically analyzed, but the mean of J78 was within the combined control range.

The insect, disease, and abiotic stressor evaluations for J3 and the Atlantic control are shown in **Table 16**. No differences were noted between J3 and the control during any of the 106 insect stressor observations, which included six different insects. For the eight disease stressors evaluated, 97 of 99 observations lacked observed differences between J3 and the control. No differences between J3 and the control were observed during any of 72 total observations of five abiotic stressors. The two observed differences between J3 and the control are detailed in the footnotes of **Table 16**. The lack of differences between J3 and the control for stressor observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

The insect, disease, and abiotic stressor evaluations for J55 and the Atlantic control are shown in **Table 16**. No differences were seen between J55 and the control during the 106 insect stressor observations including six different insects. For the eight disease stressors evaluated, 97 of 99 observations lacked observed differences between J55 and the control. No differences between J55 and the control were observed during any of 72 total observations of five abiotic stressors. The two observed differences between J55 and the control are detailed in the footnotes of **Table 16**. The lack of differences between J55 and the control for stressor observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

The insect, disease, and abiotic stressor evaluations for J78 and the Atlantic control are shown in **Table 16**. No differences were noted between J78 and the control during 106 individual observations of six insect stressors. For the eight disease stressors, 98 of 99 observations lacked observed differences between J78 and the control. No differences between J78 and the control were observed during 72 observations of five abiotic stressors. The single observed difference between J78 and the control is detailed in the footnotes of **Table 16**. The lack of differences between J78 and the control for stressor observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

Notes were taken on incidental stressors for J3, J55, J78, and the Atlantic control and are presented in **Table 17**. No incidental insect stressors were noted for J3, J78, or the control during 32 observations. One incidental insect stressor was noted for J55 out of 32 observations. Out of 66 observations, incidental *Verticillium* was noted eight times each for J3, J55, J78, and the control. No incidental virus was recorded out of 66 observations for any entry. Other incidental stressors were not found for J3, J55, J78, or the control in any of 63 observations.

It can be concluded that there are no major differences in agronomic characteristics, flower color, yield and grading, and ecological interactions between the untransformed Atlantic variety and events J3, J55, J78 (**Tables 13, 14, 15, 16, and 17**). Therefore, based on our multi-year data, the Atlantic events J3, J55, and J78 pose no significant risk of persistence in the environment as a result of weediness or plant pest potential.

Table 13. Atlantic 2010-2011 Field Trials – Agronomic Characteristics

Entry	Statistic	Characteristic (see **Table 2.** for descriptions)									
		Early Emergence	Final Emergence[1]	Stems Per Plant[2]	Plant Vigor	Foliage Color	Leaflet Size	Leaflet Curl	Senescence	Vine Size	Vine Maturity Rating
J3	Mean	71.7%	97.8%	3.4	3.2	3.1	3.1	3.0	52.4	.	2.9
	SD	23.9%	3.3%	1.34	0.78	0.36	0.45	0.00	31.31	.	0.68
	90% CI	66.0-77.3%	96.7-98.9%	2.93-3.92	3.01-3.36	3.02-3.19	3.01-3.21	3.00-3.00	39.91-64.82	.	2.71-3.07
	p-Value	0.3204	0.0879	0.0023	0.8352	0.3916	0.1360	NA	0.0044	.	NA
J55	Mean	68.6%	99.3%	3.3	3.2	3.2	3.0	3.0	52.4	.	3.0
	SD	26.2%	2.2%	1.22	0.72	0.51	0.26	0.00	30.38	.	0.94
	90% CI	62.4-74.7%	98.6-100%	2.86-3.76	2.98-3.30	3.07-3.30	2.98-3.09	3.00-3.00	40.28-64.46	.	2.73-3.22
	p-Value	0.0308	0.6324	0.0593	0.5226	0.6899	0.9144	NA	0.0044	.	NA
J78	Mean	71.8%	98.2%	3.4	3.1	3.2	3.0	3.0	51.9	.	2.7
	SD	25.9%	3.2%	1.25	0.46	0.48	0.00	0.00	30.96	.	0.85
	90% CI	65.7-77.9%	97.1-99.3%	2.89-3.80	3.02-3.22	3.07-3.28	3.00-3.00	3.00-3.00	39.58-64.21	.	2.46-2.91
	p-Value	0.3455	0.2527	0.0233	0.4170	0.7954	NA	NA	0.0094	.	NA
Atlantic Ctrl	Mean	74.3%	99.0%	3.1	3.2	3.1	3.0	3.0	47.4	.	3.0
	SD	30.4%	2.5%	1.14	0.46	0.32	0.00	0.00	32.56	.	0.11
	90% CI	67.2-81.4%	98.2-99.8%	2.72-3.55	3.06-3.27	3.04-3.19	3.00-3.00	3.00-3.00	34.42-60.32	.	3.00-3.06
ComC[3]	Range	7.0-100.0	79.0-100.0	1.6-5.0	2.3-4.3	2.3-4.0	2.0-3.0	1.0-3.0	8.7-91.7	.	2.3-4.5

[1]Final emergence data from 2011 only.

[2]Stems per plant data from 2011 only.

[3]ComC = combined control range. The range of mean values of conventional Ranger, Burbank, Atlantic, G, and H controls.

NA = Statistical comparison not possible.

Table 14. Atlantic 2011 Field Trials – Flower Color Characteristics

Entry	Number of Plots	
	Purple or Mixed Flowers	White Flowers
J3	22	0
J55	22	0
J78	22	0
Atlantic Ctrl	22	0

Table 15. Atlantic 2010-2011 Field Trials – Yield and Grading Characteristics

Entry	Statistic	Characteristic							
		Total Yield	% US#1	% Grade B	% Grade A	% Oversize	% Pick Outs[2]	Specific Gravity	Total Internal Defects
J3	Mean	42.2	84.7	14.3	79.3	5.4	1.1	1.094	30.4
	SD	15.8	7.3	7.7	7.7	7.0	1.8	0.007	23.1
	90% CI	37.5-46.9	82.5-86.9	12.0-16.6	76.9-81.6	3.3-7.5	0.5-1.6	1.092-1.096	23.5-37.3
	p-Value	0.0604	0.0175	NA	0.7763	0.0371	0.9161	0.0133	0.9572
J55	Mean	46.2	86.3	11.8	78.5	7.8	2.0	1.093	36.6
	SD	18.9	8.2	7.2	7.6	8.9	4.8	0.009	24.8
	90% CI	40.5-51.9	83.9-88.8	9.6-14.0	76.2-80.8	5.1-10.4	0.5-3.4	1.090-1.095	29.1-44.0
	p-Value	0.3913	0.4341	NA	0.8392	0.6937	0.1049	0.4442	0.1472
J78	Mean	45.4	87.4	11.5	79.6	7.9	1.0	1.092	28.9
	SD	17.0	7.3	7.4	10.2	11.8	1.9	0.008	31.3
	90% CI	40.3-50.5	85.2-89.6	9.3-13.7	76.5-82.6	4.3-11.4	0.5-1.6	1.090-1.094	19.5-38.3
	p-Value	0.7721	0.7541	NA	0.6264	0.7633	0.9580	0.6616	0.6676
Atlantic Ctrl	Mean	45.0	87.1	11.9	78.8	8.3	1.0	1.092	30.6
	SD	18.4	6.7	7.3	8.6	9.9	2.1	0.009	30.5
	90% CI	39.4-50.5	85.1-89.1	9.7-14.1	76.2-81.4	5.3-11.3	0.4-1.6	1.090-1.095	21.5-39.8
ComC[1]	Range	17.2-80.1	64.0-95.0	3.0-36.0	61.0-92.3	0.0-33.0	0.0-6.0	1.074-1.109	0.0-120.0

[1]ComC = combined control range. The range of mean values of conventional Atlantic, G, and H controls.
[2]Pick outs refer to tubers discarded at harvest due to quality issues, *i.e.* rot and mold.
NA = Statistical comparison not possible.

Table 16. Atlantic 2011 Field Trials – Stressor Observations

Stressor	J3		J55		J78	
	Total # Obs.	# Obs. Without Observed Differences*	Total # Obs.	# Obs. Without Observed Differences*	Total # Obs.	# Obs. Without Observed Differences*
Insect						
Aphids	21	21	21	21	21	21
Colorado Potato Beetle	65	65	65	65	65	65
Flea Beetle	8	8	8	8	8	8
Looper	3	3	3	3	3	3
Potato Leafhopper	6	6	6	6	6	6
Seed Corn Maggot	3	3	3	3	3	3
Totals	106	106	106	106	106	106
Disease						
Black Leg	3	3	3	3	3	3
Botrytis	24	24	24	24	24	24
Early Blight	51	50[1]	51	50[2]	51	51
Late Blight	3	3	3	3	3	3
Rhizoctonia	6	6	6	6	6	6
Verticillium	3	2[3]	3	2[4]	3	2[5]
Virus	3	3	3	3	3	3
White Mold	6	6	6	6	6	6
Totals	99	97	99	97	99	98
Abiotic						
Frost	6	6	6	6	6	6
Hail	12	12	12	12	12	12
Heat	3	3	3	3	3	3
Herbicide	21	21	21	21	21	21
Wind	30	30	30	30	30	30
Totals	72	72	72	72	72	72

*Stressor evaluations were intended to be categorical and were not statistically analyzed. The range of ratings for each event and its control were compared for each observation and an observed difference occurred when the range of the event was outside the range of the control.

[1]During one observation J3 ranged 2.5-4.5 and control ranged 3-4 (Bingham Co., ID 2011).

[2]During one observation J55 ranged 3-4.5 and control ranged 3-4 (Bingham Co., ID 2011).

[3]During one observation J3 ranged 2.5-3 and control ranged 3-3.5 (Bingham Co., ID 2011).

[4]During one observation J55 ranged 2.5-2.5 and control ranged 3-3.5 (Bingham Co., ID 2011).

[5]During one observation J78 ranged 2-3 and control ranged 3-3.5 (Bingham Co., ID 2011).

Table 17. Atlantic 2010-2011 Field Trials – Incidental Stressor Observations[1]

Entry	Incidental Insect		Incidental *Verticillium*		Incidental Virus		Incidental Other	
	Count of No	Count of Yes	Count of No	Count of Yes	Count of No	Count of Yes	Count of No	Count of Yes
J3	32	0	58	8	66	0	63	0
J55	31	1	58	8	66	0	63	0
J78	32	0	58	8	66	0	63	0
Atlantic Ctrl	32	0	58	8	66	0	63	0

[1]Specific stressors, except *Verticillium*, were not identified during the incidental stressor observations and only presence/absence data were collected. Counts are the number of observations where incidental stressors were found (yes) or looked for but not found (no).

Field Performance and Tuber Assessment for G and Event G11

Evaluations of phenotypic characteristics and ecological interactions (stressors) of the G11 event and controls grown in 2009, 2010, and 2011 are shown in **Tables 18-21**. Results were analyzed by statistical methods where possible. Overall the data suggest that there are no major differences between the G control and the G11 event.

The agronomic characteristics for G11 and the G control are shown in **Table 18**. No statistically significant differences were detected between G11 and the control for five of the six agronomic characteristics which were analyzed. Early emergence and vine maturity rating data were not able to be statistically compared. The mean values for G11 for both of these characteristics were within the combined control range. A statistically significant difference was detected between G11 and the control for plant vigor (3.5 vs. 3.0); however, the value of G11 was within the combined control range. Although not statistically significant, the values for G11 for leaflet size and vine size were outside the combined control range (3.3 vs. 2.0-3.0 and 3.3 vs. 2.1-3.0, respectively). These small observed differences are unlikely to contribute to increased weediness or pest potential.

The yield and grading characteristics of G11 and the G control are shown in **Table 19**. There were no statistically significant differences detected for % oversize, specific gravity, or total internal defects. Statistical analysis was not possible for % U.S. #1, % grade B, or % grade A; however, the value for G11 was within the combined control range for these yield and grading characteristics. Two differences were detected between G11 and the control for total yield (34.9 vs. 41.6, respectively) and % pick outs (0.5 vs. 1.8). All values for G11 for these differences were within the combined control range.

The insect and disease stressor evaluations for G11 and the G control are shown in **Table 20**. No differences were noted between G11 and the control during 44 of 48 individual observations of six insect stressors. For the single disease stressor, Verticillium, all four observations lacked observed differences between G11 and the control. The four observed differences between G11 and the control are detailed in the footnotes of **Table 20**. The lack of differences between G11 and the control for stressor observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

Notes were also taken on incidental stressors for G11 and the G control and are presented in **Table 21**. Incidental *Verticillium* was not noted for G11 or the control during 20 observations. Also, no incidental virus was found during 20 observations. Other incidental stressors were identified four times each for G11 and the control out of 24 total observations.

It can be concluded that there are no major differences in agronomic characteristics, flower color, yield and grading, and ecological interactions between the untransformed G variety and event G11 (**Tables 18, 19, 20, and 21**). Therefore, based on our multi-year data, the G event G11 poses no significant risk of persistence in the environment as a result of weediness or plant pest potential.

Table 18. G 2009-2010 Field Trials – Agronomic Characteristics

Entry	Statistic	Characteristic (see **Table 2.** for descriptions)									
		Early Emergence	Final Emergence	Stems Per Plant	Plant Vigor[1]	Foliage Color[2]	Leaflet Size[3]	Leaflet Curl[4]	Senescence	Vine Size[5]	Vine Maturity Rating[6]
G11	Mean	72.7%	90.8%	.	3.5	2.9	3.3	2.9	.	3.3	2.8
	SD	13.6%	10.0%	.	0.81	0.49	0.71	0.56	.	0.66	0.75
	90% CI	65.6-79.7%	86.4-95.2%	.	3.18-3.76	2.73-3.08	3.04-3.55	2.71-3.11	.	2.96-3.54	2.48-3.07
	p-Value	NA	0.2822	.	0.0090	0.4066	0.0582	0.4946	.	0.1495	NA
G Ctrl	Mean	77.7%	92.1%	.	3.0	3.0	3.0	3.0	.	3.0	2.8
	SD	5.0%	9.0%	.	0.05	0.05	0.00	0.05	.	0.00	0.57
	90% CI	75.1-80.2%	88.1-96.0%	.	2.97-3.01	2.97-3.01	3.00-3.00	2.97-3.01	.	3.00-3.00	2.58-3.02
ComC[6]	Range	7.0-100.0	79.0-100.0	.	2.3-4.3	2.3-4.0	2.0-3.0	1.0-3.0	.	2.1-3.0	2.3-4.5

[1]Lakeview, MI 2009 plant vigor data excluded from statistical analysis due to lack of variability.
[2]Lakeview, MI 2009 foliage color data excluded from statistical analysis due to lack of variability; foliage color data from 2010 only.
[3]Lakeview, MI 2009 leaflet size data excluded from statistical analysis due to lack of variability; leaflet size data from 2010 only.
[4]Lakeview, MI 2009 leaflet curl data excluded from statistical analysis due to lack of variability; leaflet curl data from 2010 only.
[5]Vine size data from 2010 only.
[6]ComC = combined control range. The range of mean values of conventional Ranger, Burbank, Atlantic, G, and H controls.
NA = Statistical comparison not possible.

Table 19. G 2009-2010 Field Trials – Yield and Grading Characteristics

Entry	Statistic	Characteristic							
		Total Yield	% US#1	% Grade B	% Grade A	% Oversize	% Pick Outs[2]	Specific Gravity	Total Internal Defects
G11	Mean	34.9	85.4	14.1	84.5	0.9	0.5	1.085	0.8
	SD	10.2	5.6	5.7	5.8	2.1	0.8	0.005	1.3
	90% CI	29.6-40.2	82.5-88.3	11.1-17.0	81.5-87.5	0.0-2.0	0.1-0.9	1.082-1.087	0.1-1.4
	p-Value	0.0266	NA	NA	NA	0.8499	0.0426	0.6318	0.2064
G Ctrl	Mean	41.6	82.0	16.3	80.9	1.1	1.8	1.084	0.2
	SD	8.9	8.1	6.8	7.4	2.0	2.1	0.003	0.6
	90% CI	37.0-46.2	77.8-86.2	12.8-19.9	77.1-84.7	0.1-2.1	0.8-2.9	1.082-1.086	0.0-0.5
ComC[1]	Range	17.2-80.1	64.0-95.0	3.0-36.0	61.0-92.3	0.0-33.0	0.0-6.0	1.074-1.109	0.0-120.0

[1]ComC = combined control range. The range of mean values of conventional Atlantic, G, and H controls.
[2]Pick outs refer to tubers discarded at harvest due to quality issues, i.e. rot and mold.
NA = Statistical comparison not possible.

Table 20. G 2011 Field Trials – Stressor Observations

Stressor	G11	
	Total # Obs.	# Obs. Without Observed Differences*
Insect		
Aphids	8	8
Blister Beetle	8	8
Colorado Potato Beetle	8	7[1]
Flea Beetle	8	8
Grasshopper	8	6[2]
Potato Leafhopper	8	7[3]
Totals	48	44
Disease		
Verticillium	4	4
Totals	4	4

*Stressor evaluations were intended to be categorical and were not statistically analyzed. The range of ratings for each event and its control were compared for each observation and an observed difference occurred when the range of the event was outside the range of the control.

[1]During one observation G11 ranged 4-5 and control ranged 5-5 (Oneida Co., WI 2011).

[2]During two observations G11 ranged 4-5 and 3-5 and control ranged 5-5 and 4-5, respectively (both at Oneida Co., WI 2011).

[3]During one observation G11 ranged 4-5 and control ranged 5-5 (Oneida Co., WI 2011).

Table 21. G 2010-2011 Field Trials – Incidental Stressor Observations[1]

Entry	Incidental *Verticillium*		Incidental Virus		Incidental Other	
	Count of No	Count of Yes	Count of No	Count of Yes	Count of No	Count of Yes
G11	20	0	20	0	20	4
G Ctrl	20	0	20	0	20	4

[1]Specific stressors, except *Verticillium*, were not identified during the incidental stressor observations and only presence/absence data were collected. Counts are the number of observations where incidental stressors were found (yes) or looked for but not found (no).

Field Performance and Tuber Assessment for H and Events H37 and H50

Evaluations of phenotypic characteristics and ecological interactions (stressors) of H37 and H50 events and controls grown in 2009, 2010, and 2011 are shown in **Tables 22-25**. Results were analyzed by statistical methods where possible. Overall, the data suggest that there are no major differences between the H control and the H37 and H50 events.

The agronomic characteristics for H37 and the H control are shown in **Table 22**. No statistically significant differences were detected between H37 and the control for six of the statistically analyzed agronomic characteristics. Early emergence data were not able to be statistically compared, but the mean value for H37 was within the combined control range. One statistically significant difference was detected between H37 and the control for plant vigor (3.5 vs. 3.0); however, the value of H37 was within the combined control range. Although not statistically significant, the values for leaflet size and leaflet curl were outside the combined control range (3.2 vs.2.0-3.0 and 3.1 vs. 1.0-3.0, respectively). These small observed differences would not contribute to increased weediness or pest potential.

The agronomic characteristics for H50 and the H control are shown in **Table 22**. No statistically significant differences were detected for any of the statistically analyzed agronomic characteristics. Early emergence data were not able to be statistically compared but the mean value for H50 was within the combined conventional control range. Although not statistically significant, the values for leaflet size and leaflet curl were outside the combined control range (3.3 vs.2.0-3.0 and 3.1 vs. 1.0-3.0, respectively). These small observed differences would not contribute to increased weediness or pest potential.

The yield and grading characteristics of H37 and the H control are shown in **Table 23**. There were no statistically significant differences detected for % grade A, % pick outs, or specific gravity. Statistical analysis was not possible for % U.S. #1, % grade B, or total internal defects. The value for H37 was within the combined control range for all yield and grading characteristics. Two differences were detected between H37 and the control for total yield (36.8 vs. 46.9, respectively) and % oversize (4.3 vs. 9.1). All values for H37 for these differences were within the combined control range.

The yield and grading characteristics of H50 and the H control are shown in **Table 23**. There were no statistically significant differences detected between H50 and the control for any of the analyzed yield and grading characteristics. Statistical analysis was not possible for % U.S. #1, % grade B, or total internal defects. The values for H50 were within the combined control range for all yield and grading characteristics.

The insect and disease stressor evaluations for H37 and the H control are shown in **Table 24**. No differences were noted between H37 and the control during any of the 48 individual observations of six insect stressors. For the one disease stressor evaluated, *Verticillium,* all four observations lacked differences between H37 and the control. The lack of differences between H37 and the control for stressor observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

The insect and disease stressor evaluations for H50 and the H control are shown in **Table 24**. No differences were noted between H50 and the control for 46 out of 48 observations of six insect stressors. For the single disease stressor, *Verticillium,* all four observations lacked observed differences between H50 and the control. The two observed differences between H50 and the control are detailed in the footnotes of **Table 24**. The lack of differences between H50 and the control for stressor

observations supports the conclusion that the event did not alter plant-insect, plant-disease, or plant-environment interactions.

Notes were also taken on incidental stressors for H37, H50, and the H control and are presented in **Table 25**. Incidental *Verticillium* was not noted for H37 and H control, and noted twice for H50 out of 20 total observations for each line. No incidental virus was found during 20 observations for H37, H50, or the control. Other incidental stressors were identified four times each for H37, H50, and the control out of 24 observations.

It can be concluded that there are no major differences in agronomic characteristics, flower color, yield and grading, and ecological interactions between the untransformed H variety and events H37 and H50 (**Tables 22, 23, 24, and 25**). Therefore, based on our multi-year data, the H events H37 and H50 pose no significant risk of persistence in the environment as a result of weediness or increased plant pest potential.

Table 22. H 2009-2010 Field Trials – Agronomic Characteristics

Entry	Statistic	Characteristic (see **Table 2.** for descriptions)									
		Early Emergence	Final Emergence	Stems Per Plant	Plant Vigor[1]	Foliage Color[2]	Leaflet Size[3]	Leaflet Curl[4]	Senescence	Vine Size[5]	Vine Maturity Rating
H37	Mean	67.0%	88.3%	.	3.5	3.0	3.2	3.1	.	2.9	3.1
	SD	10.5%	10.7%	.	0.80	0.52	0.80	0.46	.	0.78	0.77
	90% CI	61.5-72.5%	83.6-93.0%	.	3.15-3.77	2.83-3.23	2.92-3.54	2.96-3.31	.	2.56-3.25	2.78-3.37
	p-Value	NA	0.1554	.	0.0222	0.8207	0.1434	0.2844	.	0.4534	0.0617
H50	Mean	73.3%	92.1%	.	3.2	3.0	3.3	3.1	.	2.8	3.1
	SD	8.4%	8.4%	.	0.80	0.49	0.62	0.46	.	0.75	0.95
	90% CI	69.0-77.7%	88.4-95.7%	.	2.85-3.47	2.82-3.20	3.03-3.51	2.95-3.31	.	2.45-3.11	2.73-3.47
	p-Value	NA	0.9371	.	0.4187	0.9226	0.0859	0.2994	.	1.0000	0.0913
H Ctrl	Mean	77.0%	91.9%	.	3.0	3.0	3.0	3.0	.	2.8	3.3
	SD	4.9%	9.2%	.	0.00	0.00	0.00	0.00	.	0.41	0.67
	90% CI	74.5-79.5%	87.8-95.9%	.	3.00-3.00	3.00-3.00	3.00-3.00	3.00-3.00		2.60-2.96	3.06-3.59
ComC[6]	Range	7.0-100.0	79.0-100.0	.	2.3-4.3	2.3-4.0	2.0-3.0	1.0-3.0	.	2.1-3.0	2.3-4.5

[1]Lakeview, MI 2009 plant vigor data excluded from statistical analysis due to lack of variability.
[2]Lakeview, MI 2009 foliage color data excluded from statistical analysis due to lack of variability; foliage color data from 2010 only.
[3]Lakeview, MI 2009 leaflet size data excluded from statistical analysis due to lack of variability; leaflet size data from 2010 only.
[4]Lakeview, MI 2009 leaflet curl data excluded from statistical analysis due to lack of variability; leaflet curl data from 2010 only.
[5]Vine size data from 2010 only.
[6]ComC = combined control range. The range of mean values of conventional Ranger, Burbank, Atlantic, G, and H controls.
NA = Statistical comparison not possible.

Table 23. H 2009-2010 Field Trials – Yield and Grading Characteristics

Entry	Statistic	Characteristic							
		Total Yield	% US#1	% Grade B	% Grade A	% Oversize	% Pick Outs[2]	Specific Gravity	Total Internal Defects
H37	Mean	36.8	88.3	11.4	83.8	4.3	0.5	1.075	1.6
	SD	8.5	5.2	5.1	7.1	5.9	0.9	0.005	1.5
	90% CI	32.4-41.2	85.6-90.9	8.8-14.0	80.2-87.5	1.3-7.4	0.0-1.0	1.072-1.077	0.8-2.4
	p-Value	0.0425	NA	NA	0.2460	0.0048	0.1456	0.0777	NA
H50	Mean	47.3	91.2	7.8	83.1	8.0	1.2	1.076	3.7
	SD	16.7	4.4	4.0	8.0	7.4	1.2	0.004	2.1
	90% CI	38.6-56.0	88.9-93.4	5.7-9.8	78.9-87.2	4.2-11.8	0.5-1.8	1.074-1.078	2.6-4.8
	p-Value	0.9309	NA	NA	0.4886	0.4818	1.0000	0.4950	NA
H Ctrl	Mean	46.9	91.2	7.8	82.0	9.1	1.2	1.077	1.8
	SD	8.9	5.0	4.4	8.3	9.0	1.6	0.004	1.2
	90% CI	42.2-51.5	88.6-93.7	5.5-10.0	77.7-86.3	4.4-13.8	0.3-2.0	1.075-1.079	1.1-2.4
ComC[1]	Range	17.2-80.1	64.0-95.0	3.0-36.0	61.0-92.3	0.0-33.0	0.0-6.0	1.074-1.109	0.0-120.0

[1]ComC = combined control range. The range of mean values of conventional Atlantic, G, and H controls.
[2]Pick outs refer to tubers discarded at harvest due to quality issues, i.e. rot and mold.
NA = Statistical comparison not possible.

Table 24. H 2011 Field Trials – Stressor Observations

Stressor	H37		H50	
	Total # Obs.	# Obs. Without Observed Differences*	Total # Obs.	# Obs. Without Observed Differences*
Insect				
Aphids	8	8	8	8
Blister Beetle	8	8	8	7[1]
Colorado Potato Beetle	8	8	8	8
Flea Beetle	8	8	8	8
Grasshopper	8	8	8	7[2]
Potato Leafhopper	8	8	8	8
Totals	48	48	48	46
Disease				
Verticillium	4	4	4	4
Totals	4	4	4	4

*Stressor evaluations were intended to be categorical and were not statistically analyzed. The range of ratings for each event and its control were compared for each observation and an observed difference occurred when the range of the event was outside the range of the control.

[1]During one observation H50 ranged 4-5 and control ranged 5-5 (Oneida Co., WI 2011).

[2]During one observation H50 ranged 3-5 and control ranged 4-5 (Oneida Co., WI 2011).

Table 25. H 2010-2011 Field Trials – Incidental Stressor Observations[1]

Entry	Incidental *Verticillium*		Incidental Virus		Incidental Other	
	Count of No	Count of Yes	Count of No	Count of Yes	Count of No	Count of Yes
H37	20	0	20	0	20	4
H50	18	2	20	0	20	4
H Ctrl	20	0	20	0	20	4

[1]Specific stressors, except *Verticillium*, were not identified during the incidental stressor observations and only presence/absence data were collected. Counts are the number of observations where incidental stressors were found (yes) or looked for but not found (no).

MATERIALS AND METHODS

Tuber Seed. The 2009, 2010, and 2011 trials were planted mechanically to facilitate harvests and ensure that the regulated potatoes were kept separate from unmodified material. To explain the terminology used for multiple generations of tuber seed, the following describes a typical seed bulk-up program. In the initial year, propagation in tissue culture would produce about 100 plants, and then these plants are grown in a greenhouse to produce thousands of mini-tubers, also called "nuclear seed". In the second year, mini-tubers are planted at field sites with the lowest possible incidence of diseases and pests, producing generation-1 (G1) seed. Those G1 tubers are grown in the third year to increase the amount of tubers, producing G2 seed. In the fourth, fifth, and sixth years, bulk-up continues with commercial seed often representing G3, G4, and G5 tubers respectively.

For the 2009 evaluations, "nuclear seed" mini-tubers from each event and the control varieties were used to plant four or five single-row plots (20 mini-tubers/plot) whereby the plots were randomly distributed within blocks across the field (**Table 1**). This randomized complete block design (RCB) is typical for the evaluation of new potato varieties and events. The approach taken in 2010 and 2011 was to use three random plots per event and control per site, also using the RCB design with the number or replications (plots per event) equal to the number of blocks. Each plot in 2010 consisted of three rows of 20 seed pieces each from first generation (G1) tubers produced in Cherry County, NE, in 2009 for Ranger Russet and Russet Burbank, and from G1 tubers produced in Oneida County, WI, in 2009 for the G and H varieties (**Table 1**). The Atlantic trials of 2010 were still based on the use of nuclear seed mini-tubers. The Ranger Russet and Russet Burbank seed for the 2011 trials was second generation (G2) produced in Cherry County, NE in 2010, except for F37 which was planted with "nuclear seed" mini-tubers. The Atlantic seed was first generation (G1) also produced in Cherry County, NE in 2010. In 2010 and 2011, the G and H seed was first generation (G1) and second generation (G2), respectively produced in Oneida County, WI in 2009 and 2010. In all trials, the seed of the events were handled similarly to the untransformed controls.

Field grown tubers are more desirable than mini-tubers as seed because they generate more vigorous and uniform plants that produce higher tuber yield and quality. Nuclear seed mini-tubers (10-30 mm size) were produced from pathogen-free tissue culture parent stock using a proprietary hydroponic system under controlled greenhouse conditions. G1 seed pieces were obtained by harvesting pathogen-free potato tubers produced from the nuclear seed and then cutting them, either mechanically or by hand, into 2-4 oz. seed pieces.

In-row seed spacing varied between 8-12 inches depending on the commercial standard for the variety and region. Planting depth of mini-tubers varied between 2-3 inches, and for field grown seed pieces between 7-12 inches, dependent on both the planting material used and the environmental conditions.

Emergence. Sprout surfacing during the early season (May-June for most trials except for the Florida trials, which were evaluated in March) was evaluated by determining the frequency of plants that emerged from either mini-tubers (in 2009 for all varieties) or seed pieces (in 2010 for most varieties except for Atlantic, and in 2011 for all varieties). Emergence rates vary among conventional varieties and are dependent on both the physiological age of the "seed" and environmental factors such as temperature and moisture levels.

Phenotypic Assessments. Each plot was evaluated qualitatively, in some cases using standardized monitoring scales, for differential responses to insect, disease, and environmental stresses that were not induced artificially, but might occur spontaneously during the growing season. Mid-season monitoring was conducted 2009, 2010, and 2011 just prior to or during early row closure and flowering (June-July for most trials except for the Florida trials, which were evaluated in April), to assess plant vigor, leaf color, leaf size, leaf curl, disease symptoms (presence/absence), and insect-associated plant damage. In 2011, specific insects, diseases, and abiotic stressors common to the growing region were evaluated. The 1 to 5 rating scale, which is used routinely by breeders to rate plants in potato variety evaluations, is explained in **Table 2.** Disease and insect pressure are generally highest during the mid and late season, so the plants were monitored for symptoms caused by the pathogens and insects such as those listed in **Table 26** from July to September (March through May for Florida trials). Not all insects or diseases were evaluated or found at each site. Late season monitoring of vine maturity and diseases was performed once prior to vine killing, a process intended to ensure tuber maturation and late-season skin set. Vine killing is induced either by mowing or flailing the vines or by using approved herbicides such as Reglone® according to the manufacturer's recommendations (JR Johnson, Roseville, MN). At this time, plants were also assessed for disease symptoms and insect damage. In some cases when disease symptoms were identified, a sprout test was also conducted to confirm the findings. The events were evaluated for susceptibility to *Phytophthora infestans* and *Erwinia carotovora*, and this data can be found in **Appendix 8. Disease Susceptibility**. To summarize the insect, disease, and abiotic stressor ratings, the individual observations for each stressor were totaled across time points and sites. The total number of observations includes observations where a stressor was found and rated as well as observations where a stressor was looked for but not found (i.e., rating = 0). When the range of insect, disease, or abiotic stressor ratings for the event did not overlap with the control they were considered different.

Table 26. Common Potato Disease and Insect Symptoms[1]

Insect or Disease Agent	Symptom
Emposasca fabae (Potato Leafhopper)	Leaf feeding damage
Epitrix species (Flea Beetle)	Shot-holes in leaves
Leptinotarsa decemlineata (Colorado Potato Beetle)	Defoliation
Limonius californicus (Wireworm)	Bored holes in tubers and shoots
Ostrinia nubilalis (European Corn Borer)	Severe vine wilting above point of injury
Bactericera (Paratrioza) cockerelli (Potato Psyllid)	Yellows
Phthorimaea operculella (Tuberworm)	Foliar and tuber damage
Various aphid spp.	Leaf suckling damage
Aster Yellows MLO	Purple top disease
Potato Leafroll Virus	Rolling of leaves and net necrosis
Potato Spindle Tuber Viroid	Potato spindle tuber disease
Potato Virus A,M, X, Y	Mosaic symptoms
Tobacco Rattle Virus	Stem mottling
Erwinia carotovora	Blackleg, aerial stem rot and tuber soft rot
Corynebactium sepedonicum	Bacterial ring rot
Ralstonia solanacearum	Brown rot
Phytophthora infestans	Late blight
Phytophthora erythroseptica	Pink rot
Verticillium spp.	Early dying
Sclerotinia sclerotiorum	Sclerotinia stalk rot
Rhizoctonia solani	Canker
Streptomyces scabies	Scab
Fusarium spp.	Dry rot
Pythium ultimum	Water rot, shell rot, *Pythium* leak
Alternaria solani	Early blight
Botrytis cinerea	Gray mold

[1]Not necessarily observed at all sites or observation timings. This table is meant to give the reader an accurate list of insects and diseases that may impact potatoes.

Tuber Grading. The grading methods employed were identical to those used to grade commercial potatoes intended for the production of fries or chips.

Tubers were harvested during early fall except for trials in St Johns County, FL, where tubers were harvested during late-spring. At each site in 2009, approximately 16 lbs. of tubers were collected from each plot and replicates were pooled for each entry. In 2010 and 2011, samples were taken from each plot and kept separate. Harvested material was stored for two to six weeks in temperature and humidity controlled facilities at 50-55^{O} F and 95%, respectively. Material from the St Johns County, FL, trial was harvested, graded on site, and then shipped immediately for evaluation.

Yields were determined by weighing the total amount of potatoes harvested from each plot. Samples of tubers were graded for characteristics listed in **Table 2** at (a) the State of Idaho Department of Agriculture Grading Facility in Caldwell, ID, for Ranger Russet and Russet Burbank, (b) Michigan State University by Dr. David Douches for Atlantic, and (c) the facilities of an industry partner for the G and H varieties. Tubers were considered to have internal defects if they had at least one of the following: hollow heart, vascular ring discoloration, heat/internal brown spot, brown center, net necrosis and insect damage. Percentages of each individual defect were summed and these data are presented in the results as total internal defects.

Size profiles of Ranger Russet and Russet Burbank were determined by weighing tubers from a subset of the sample (50-60 lbs.) sorted in batches of 4, 6, 10 and 14 oz. Six-ounce tuber size produces optimal

French fries and allows the French fry processor to meet most customer specifications at a highest potential recovery for fry production. For the Atlantic, G, and H lines, tubers were categorized as U.S. #1, Grade B, Grade A, oversize, or pick out. These categories are appropriate for chipping varieties.

The specific gravity of tuber lots was determined by evaluating approximately 8 lbs. of tubers from each sample using a weight in air/weight in water measurement. The sub-samples of tubers were first weighed in air and then weighed submerged under water at room temperature. From the two measurements, specific gravity was calculated using the following formula: specific gravity = weight in air/ (weight in air - weight in water). Specific gravity is the industry standard for measuring solids and is thus an important characteristic to compare the events to the conventional controls.

Statistical Methods. Means, standard deviations, and 90% confidence intervals were calculated using JMP 9.0.2. A combined control range was generated by obtaining the minimum and maximum mean values (year*location*entry) of all conventional varieties included in the experiments.

All characteristics for the Ranger Russet, Russet Burbank, Atlantic, G, and H varieties were analyzed in JMP 9.0.2 by combining data from multiple years and locations using the following linear mixed model:

$$Y_{ijkm} = \alpha_{i+}\beta_j + \gamma_{k(j)} + \delta_{l(j,k)} + (\alpha\gamma)_{ik} + \varepsilon_{ijklm}$$

- α = mean of treatment (fixed)
- β = year (random)
- γ = effect of location [year] (random)
- δ = rep[year, location] (random)
- ε = residual random error

$\beta_j \sim iidN(0, \sigma^2_{year})$, $\gamma_{k(j)} \sim iidN(0, \sigma^2_{loc(year)})$, $\delta_{l(j,k)} \sim iidN(0, \sigma^2_{rep(year, loc)})$, $(\alpha\gamma)_{ik} \sim iidN(0, \sigma^2_{loc*treatment})$, and $\varepsilon_{ijkm} \sim iidN(0, \sigma^2)$

(This means that all error terms are assumed to be normally distributed with a mean of 0 and equal variances).

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), $\gamma_{k(j)}$ 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.

The difference between LS means of test lines and the LS means of corresponding control lines was compared using contrasts. A significant difference was established with a p-value < 0.05. Every effort was made to generate p-values to aid in the interpretation of the data. Some departures from the assumptions of normality and equal variances were allowed since the results were always interpreted in the context of variation observed in the combined control range. In some cases, it was necessary to omit data from the analysis as noted in the data tables. In a few cases, generating a p-value was not possible and 'NA' was reported.

For characteristics where data were available only from a single year the following linear mixed model was used instead:

$$Y_{ijkm} = \alpha_{i +} \beta_j + \gamma_{k(j)} + (\alpha\ \beta)_{ij} + \varepsilon_{ijkm}$$

- α = mean of treatment (fixed effect)
- β = effect of location (random)
- γ = rep within location effect (random)
- ε = residual random error

$\beta_j \sim iidN(0,\sigma^2_{loc})$, $\gamma_{k(i)} \sim iidN(0, \sigma^2_{loc(rep)})$, $(\alpha\ \beta)_{ij} \sim iidN(0, \sigma^2_{loc*treatment})$, and $\varepsilon_{ijkm} \sim iidN(0, \sigma^2)$

(This means that all error terms are assumed to be normally distributed with a mean of 0 and equal variances).

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_{ijkm} denotes the residual random error.

CONCLUSIONS

Although some significant agronomic differences were observed between events and controls, most fell within the combined control range of the conventional varieties. For example, some differences in yield and tuber size were observed in the events, and these attributes will be investigated further in commercial trials. Thus, we concluded that no biologically meaningful differences that would contribute to increased weediness or pest potential were observed for any of the agronomic characteristics, yield and grading characteristics, or ecological interactions (namely plant-insect interactions, plant-disease interactions, and plant interactions with abiotic stressors) of the events compared to their untransformed controls. There were no meaningful differences in phenotype and tuber composition (**Appendix 9. Compositional Analyses**) or disease susceptibility (**Appendix 8. Disease Susceptibility**). It can be concluded that events are not different from their untransformed controls in terms of agronomy, tuber yield, and tuber composition. Therefore, the transformation of potato with pSIM1278 does not introduce characteristics that will encourage or enhance weediness or pest potential.

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.

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Appendix 7

Black Spot Bruise Tolerance

INDEX

INTRODUCTION

Black spot bruise is a discoloration affecting bruised tubers that represents one of the most important quality issues for the potato industry. The condition is a result of leakage of polyphenol oxidase (Ppo) from damaged plastids into the cytoplasm. In the cytoplasm, the enzyme oxidizes polyphenol, which then form dark precipitants. One of the two silencing cassettes of the pSIM1278 DNA insert contains two copies of a fragment of the *Ppo5* gene from *Solanum verrucosum* positioned as an inverted repeat between regulatory elements. Expression of this inverted repeat triggers silencing of the potato *Ppo5* gene. The variety Atlantic is different from other potatoes in that it doesn't express a *Ppo5* gene in high enough levels to blacken wounded tubers, and therefore was omitted from these tests.

Field-grown tubers from the seven events derived from the black spot bruise susceptible varieties; Ranger Russet, Russet Burbank, G, and H, were assayed in two ways for black spot bruise tolerance.

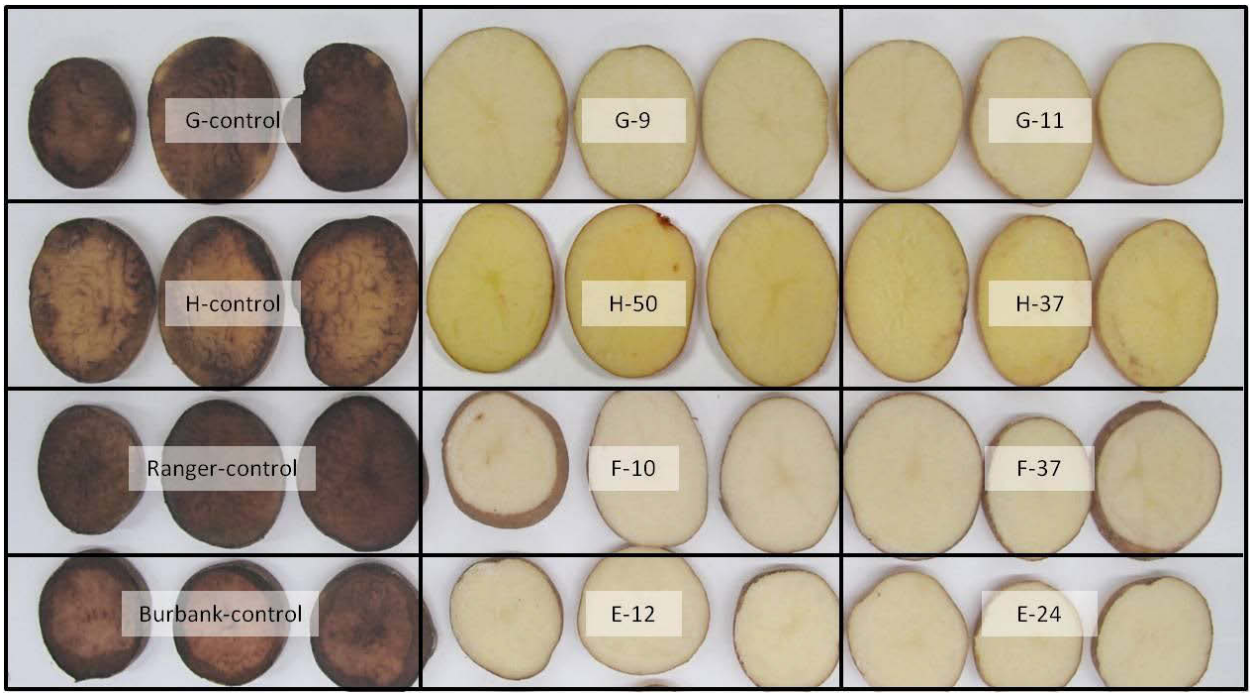
Tuber discoloration is caused by the activity of polyphenol oxidase, which oxidizes phenols including catechol to produce compounds that rapidly polymerize to produce pigments. An indirect method to test for black spot bruise tolerance is based on pipetting 1-ml catechol (25 mM in 50 mM MOPS, pH6.5) onto the cut surfaces of tubers and monitoring for the Ppo-dependent development of a dark brown precipitate. Tubers for Ranger Russet and Russet Burbank controls and events were harvested from three different plants that grew in Canyon County, ID, in 2009, and G and H controls and events were obtained from independent plants from Oneida County, WI. The assay was not applied to Atlantic (and the Atlantic events J3, J55, and J78) because Atlantic does not brown like the other varities when exposed to catechol, and shows some tolerance to black spot bruise.

The second method of assaying for bruise tolerance was by treating nine tubers of each event to 10 revolutions in a bruise barrel (rotating drum), incubating at room temperature for 3 days, followed by peeling mechanically, and then counting the number of both blackspot bruise and lineshatter (shatter bruise) spots. Tubers were from Canyon County, ID, for Ranger Russet and Russet Burbank controls and events; and G and H varieties were from Oneida County, WI, or Montcalm County, MI. All were harvested in 2009.

RESULTS

Tubers for Ranger Russet and Russet Burbank controls and events were harvested from three different plants that grew in Canyon County, ID, in 2009 (due to limitations of tuber material), and G and H controls and events were obtained from independent plants from Oneida County, WI, in 2009. An analysis of tubers demonstrated that none of them had detectable levels of functionally-active polyphenol oxidase, indicating that they display black spot bruise tolerance (**Figure 1**).

Figure 1. Catechol assay for polyphenol oxidase activity*



*Event G9 is not included in this submission for deregulation.
Note: Slight differences in flesh color of the event tubers is from natural variation and is not related to *Ppo* silencing. Variety H naturally has pale yellow flesh.

Results from the bruise barrel test (**Table 1)** show that tubers of three events (F10, F37, E12) were more resistant to black spot bruise than tubers of their untransformed control varieties (P<0.05). Numerical data indicated a trend for improved tolerance in the events, E24, H37, and H50, although this was not always statistically significant. Data for G11 were different from control in Michigan, but not in Wisconsin, suggesting that environmental factors may play an important role in efficacy of the technology for this particular genotype. For instance, certain stress factors might induce expression of an alternative *Ppo* gene in this variety.

Table 1. Bruise Barrel Test for Black Spot Bruise Tolerance

Variety, Location - 2009	No. of bruises per tuber[1]	Difference from control (P-value)
Ranger Russet Control (Canyon County, ID)	4.56 ± 1.81	
F10 (Canyon County, ID)	0.89 ± 0.78	P < 0.0001
F37 (Canyon County, ID)	0.78 ± 0.83	P < 0.0001
Russet Burbank Control (Canyon County, ID)	0.50 ± 0.79	
E12 (Canyon County, ID)	0.00 ± 0.00	P = 0.0107
E24 (Canyon County, ID)	0.28 ± 0.57	P = 0.3396
Atlantic Control (Canyon County, ID)	NA	
J3 (Canyon County, ID)	NA	
J55 (Canyon County, ID)	NA	
J78 (Canyon County, ID)	NA	
G Control (Oneida County, WI)	2.50 ± 1.07	
G11 (Oneida County, WI)	3.44 ± 0.88	P = 0.64
G Control (Montcalm County, MI)	0.78 ± 0.83	
G11 (Montcalm County, MI)	0.11 ± 0.33	P = 0.041
H Control (Oneida County, WI)	2.67 ± 1.80	
H37 (Oneida County, WI)	1.87 ± 0.641	P = 0.26
H50 (Oneida County, WI)	1.56 ± 0.73	P = 0.11
H Control (Montcalm County, MI)	2.56 ± 1.67	
H37 (Montcalm County, MI)	0.75 ± 0.886	P = 0.015
H50 (Montcalm County, MI)	1.78 ± 1.20	P = 0.273

[1]Data represent the mean of 9 tubers ± standard error.
NA= not applicable; Atlantic controls and events appeared less susceptible to black spot bruise and were not included in these tests.

DISCUSSION

Two different methods were utilized to measure the incidence of black spot bruise in potato events expressing the pSIM1278 construct. They demonstrate that transformation of the black spot bruise sensitive varieties Ranger Russet, Russet Burbank, G and H with the DNA insert of pSIM1278 results in at least partial tolerance against impact-induced discoloration. The Atlantic variety lacks a highly expressed *Ppo* gene in tubers and shows some tolerance to black spot bruise, thus silencing with pSIM1278 would not affect expression as much as with other varieties. In our construct, we targeted the *Ppo5* gene that is predominantly expressed in mature tubers and stolons (Hunt *et al.* 1993; Thygesen *et al.*1995). As expected based on the specificity of the *Ppo5* gene and use of the Agp and Gbss promoters, which are highly active in tubers and stolons (Nakata *et al.* 1994; Visser *et al.* 1991), we confirmed the predicted reduction in black spot bruise as also shown in **Appendix 5 (Efficacy and Tissue-Specificity of Gene Silencing)**.

MATERIALS AND METHODS

Materials for polyphenol oxidase assay with catechol. Tubers for Ranger Russet and Russet Burbank controls and events were harvested from three different plants that grew in Canyon County, ID, in 2009, and G and H controls and events were obtained from independent plants from Oneida County, WI, in 2009. The assay was not applied to Atlantic (and the Atlantic events J3, J55, and J78) because cut tubers from this variety do not develop a brown color when exposed to catechol.

Polyphenol oxidase activity assay. 1-ml catechol (25 mM in 50 mM MOPS, pH6.5) was pipetted onto the cut surfaces of three randomly chosen tubers for each event and control. The Ppo-dependent development of a dark brown precipitate was assessed after 20 min.

Bruise barrel test. Nine tubers of each event were subjected to 10 revolutions in a bruise barrel (rotating drum), incubating at room temperature for 3 days, followed by peeling mechanically, and then counting the number of both blackspot bruise and lineshatter (shatter bruise) spots. Tubers were from Canyon County, ID, for Ranger Russet and Russet Burbank controls and events; and G and H varieties were from Oneida County, WI, or Montcalm County, MI. All were harvested in 2009.

Statistics. Data are presented as the means of the results of three experiments, and the error bars shown represent the standard error of the mean. Significance was determined using the Student's two-tailed *t*-test ($p < .05$).

CONCLUSIONS

Russet Event F10: Both the catechol assay and barrel test confirmed that event F10 is more resistant to black spot bruise than its untransformed control.

Ranger Russet Event F37: Both the catechol assay and barrel test confirmed that event F37 is more resistant to black spot bruise than its untransformed control

Russet Burbank Event E12: Both the catechol assay and barrel test confirmed that event E12 is more resistant to black spot bruise than its untransformed control

Russet Burbank Event E24: Results of the catechol assay confirmed silencing of the polyphenol oxidase gene in tubers. In addition, the mean value for E24 with the barrel test was lower than controls, yet this difference was not statistically significant.

Atlantic Events J3, J55, and J78: These events were not tested for black spot bruise because the Atlantic variety showed some resistance to darkening both with the catechol assay and with impact in the barrel test.

G event G11: Results of the catechol assay confirmed silencing of the polyphenol oxidase gene in tubers. In addition, the barrel test showed a significant reduction in tubers from one location but no difference from control in a second test from another location.

H event H37: Results of the catechol assay confirmed silencing of the polyphenol oxidase gene in tubers. In addition, the barrel test showed a significant reduction in tubers from one location but no difference from control in a second test from another location.

H event H50: Results of the catechol assay confirmed silencing of the polyphenol oxidase gene in tubers. In addition, mean values for H50 with the barrel test were lower than controls, yet the differences were not statistically significant.

An evaluation of data presented in this appendix, together with those described in **Appendix 5. Efficacy and Tissue-Specificity of Gene Silencing**, **Appendix 6. Field Performance and Tuber Evaluations**, **Appendix 8. Disease Susceptibility**, and **Appendix 9. Compositional Analyses** demonstrate that the enhanced black spot bruise resistance in the Russet Burbank, Ranger Russet, G, and H events did not change the agronomic characteristics, disease susceptibility and compositional profile of potato.

REFERENCES

Hunt MD, Eannetta NT, Yu H, Newman SM, Steffens JC (1993) cDNA cloning and expression of potato polyphenol oxidase. Plant Mol Biol, 21: 59-68.

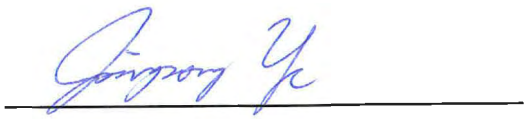
Nakata PA, Anderson JM, Okita TW (1994) Structure and expression of the potato ADP-glucose pyrophosphorylase small subunit. J Bio Chem 269: 30798-30807.

Thygesen PW, Dry IB, Robinson SP (1995) Polyphenol oxidase in potato. A multigene family that exhibits differential expression patterns. Plant Physiol, 109: 525-531.

Visser RGF, Stolte A, Jacobsen E (1991) Expression of a chimeric granule-bound starch synthase-GUS gene in transgenic potato plants. Pant Mol Bio, 691-699.

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.



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Appendix 8
Disease Susceptibility

INDEX

INTRODUCTION

The purpose of this study was to determine how common potato diseases affect the events, compared with the untransformed controls. Researchers have investigated the relationship between PPO and disease resistance (Valentines *et al*. 2005; Li and Steffens 2002; Hakimi *et al*. 2006); with some proposing that enhanced PPO may increase resistance to disease, while others claim that reduced PPO could increase resistance. Considering that some evidence exists for a relationship between PPO and diseases, we chose to test the events by infecting foliage and tubers with the causal agents of the most important oomycete (*Phytophthora infestans*) and bacterial soft rot (*Erwinia carotovora*) diseases.

Late blight tests were conducted using *Phytophthora infestans* strains US8, US10, and US22. Of these three strains, US8 and US10 were commonly found in Michigan (Young *et al.* 2009), where some of our testing was conducted. Foliar studies in 2011 used US22, a relatively new genotype that has been prevalent in the Midwestern US (Gevens *et al.* 2011).

RESULTS

Tuber Susceptibility to Late Blight

To determine the extent of symptom development in tubers infected with the causal agent of late blight, *P. infestans*, we selected four each of five field-grown tubers from Canyon County, ID, for Ranger Russet and Russet Burbank controls and events, and Oneida County, WI, for the G and H controls and events harvested during the fall of 2009.

For this analysis, infected tubers were scored on a 0-9 scale for disease symptoms 2 months after inoculation. A zero score meant there were no visible late blight disease symptoms; that is, no tuber discoloration, no surface mycelium and no physical tissue breakdown. A score of 9 was indicative of 100% late blight infection manifested by mycelium on the entire tuber surface and either with or without tissue discoloration and breakdown.

Table 1. Late Blight Symptoms in Tubers of Ranger Russet and Russet Burbank Events[1]

Line	Rating[2] (Mean) +/- Standard Error[3]
Russet Burbank Control	1.05 a +/- 0.263
E12	3.85 b +/- 0.263
E24	1.90 a +/- 0.580
Ranger Russet Control	5.60 a +/- 1.324
F10	0.80 b +/- 0.216

[1]Data in this table are from tubers harvested in 2009.

[2]Disease severity scored on 0-9 scale with 9 being 100% tuber infection with *P. infestans* strain US8. Pairwise comparisons to the controls were made using Tukey-Kramer HSD. Different letters from control show significant differences (P < 0 .05).

[3]Standard Error uses a pooled estimate of error variance. Within variety, means with the same letter are not significantly different.

As shown in **Table 1**, the Russet Burbank event E24 did not differ from the control whereas E12 appeared to be more susceptible. Although we observed more susceptibility in this laboratory study, we would not consider this one study as definitive, and therefore repeated the assay in 2011 (see results in **Table 3**).

In the assay of tubers harvested in 2009 (**Table 1)**, the Ranger Russet event F10 developed less disease symptoms upon infection than the untransformed control. In fact, event F10 was about as tolerant to late blight as the Russet Burbank control.

The G and H controls and events were tested for susceptibility to three different important U.S. strains of *P. infestans*. Results were variable, with some of the events more or less susceptible than controls, depending on the strain (**Table 2**). Event G11 was less susceptible to US8 and US22 than control G. Both H37 and H50 were more susceptible to US22 than H control, however H37 was less susceptible and H50 more susceptible to US8. There were no differences observed between events or controls tested with US10. While these laboratory tests could indicate if there were clear differences in susceptibility, with mixed results, we are unable to draw definitive conclusions.

Table 2. Late Blight Symptom in Tubers of G and H Events[1,2]

Line	Rating[2] (Mean) +/- Standard Error[3] (US8)	Rating[2] (Mean) +/- Standard Error[3] (US10)	Rating[2] (Mean) +/- Standard Error[3] (US22)
G Control	8.26 a +/- 1.693	1.68 a +/- 0.442	3.26 a +/- 0.872
G11	2.91 b +/- 1.198	3.63 a +/- 1.302	0.67 b +/- 0.281
H Control	1.69 a +/- 0.510	3.65 a +/- 1.073	1.68 a +/- 0.447
H37	0.57 b +/- 0.308	3.43 a +/- 1.041	4.91 b +/- 1.331
H50	4.21 b +/- 1.325	5.39 a +/- 1.650	4.39 b +/- 1.537

[1]Data in this table are from tubers harvested in 2009.
[2]Disease severity scored on 0-9 scale with 9 being 100% tuber infection with a specific strain of *P. infestans* (US8, US10, or US22). Different letters from control show significant differences (P < 0 .05) using Tukey-Kramer HSD.
[3]Standard Error uses a pooled estimate of error variance. Within variety, means with the same letter are not significantly different.

As a follow-up to late blight testing with laboratory assays in 2009, we conducted an additional laboratory assay in 2011, only for the Ranger Russet, Russet Burbank, and Atlantic varieties and events In this study, tubers were inoculated with the US8 strain of *P. infestans,* and progression in the events and untransformed controls were analyzed. The results of that study are summarized in **Table 3**. For both the tuber and foliar assays (Tables 3 and 4) the Russet Burbank, Ranger Russet, and Atlantic lines received priority over the proprietary G and H lines in 2011 studies. However, all events had some testing for late blight and *Erwinia* over the three years of tesing from 2009 to 2011.

Table 3. Late Blight Symptoms in Tubers of Ranger Russet, Russet Burbank and Atlantic Events[1]

Line	Avg. Disease %	P-value[2]
Russet Burbank Control	74.50	
E12	23.50	0.0296
E24	31.00	0.0637
Ranger Russet Control	65.50	
F10	71.65	0.6466
F37	57.25	0.4756
Atlantic Control	11.50	
J3	73.50	0.0024
J55	43.60	0.0919
J78	36.55	0.2181

[1]Data in this table are from tubers harvested in 2011.
[2]P-values were determined with Tukey-Kramer HSD analyses with a significance level of P < 0.05. P-values indicating significant differences with controls are underlined.

For tubers harvested in 2011, events and controls were evaluated by scoring percent late blight infection of the tuber (**Table 3**). A score of 0% would mean that there were no visible late blight disease symptoms; that is, no tuber discoloration, no surface mycelium and no physical tissue breakdown. A score of 100% late blight infection would be indicated by mycelium on the entire tuber surface, either with or without tissue discoloration and breakdown.

As shown in **Table 3**, event E12 showed significantly fewer late blight symptoms than the untransformed Russet Burbank control. This lower infection rate in E12 compared with Russet Burbank control did not confirm the earlier lab test in 2009 that suggested that E12 was more susceptible. No differences were observed between the infection of Ranger Russet Control compared with events F10 or F37. The event J3 displayed significantly more tuber infection symptoms than the Atlantic control, but J55 and J78 were not significantly different. The susceptibility of J3 tubers to late blight infection was similar to that observed in Ranger and Burbank controls, and could be effectively managed by applying standard agricultural practices in regions prone to late blight (mainly the Northeastern part of the United States).

Foliar Susceptibility to Late Blight

To determine the extent of symptom development in foliage infected with the causal agent of late blight, *Phytophthora infestans*, we conducted a RCBD field trial consisting of 3 replications of 5-hill plots in 2011. The foliage was inoculated with genotype US22, another important U.S. strain of late blight, at approximately 54 days after planting, and the percent of foliar infection was recorded at numerous times during the season (**Table 4**).

Table 4. Late Blight Symptoms in Foliage of Ranger Russet, Russet Burbank and Atlantic Events[1]

Line	RAUDPC	P-value[2]
Burbank Control	21.9	
E12	19.9	0.8361
E24	19.7	0.7963
Ranger Control	24.3	
F10	17.3	<0.0001
Atlantic Control	33.6	
J3	35.8	0.6929
J55	33.7	0.9999
J78	32.0	0.8686

[1]Data in this table are from 2011 disease analyses.

[2]P-values determined with Tukey-Kramer HSD analyses with a significance level of $P < 0.05$. P-values indicating significant differences with controls are underlined.

Relative Area Under the Disease Progress Curve (RAUDPC) was calculated from data collected at eight different time-points during the season, and used to evaluate disease severity. As presented in **Table 4**, the foliage of the events did not show increased susceptibility to late blight. Rather, the Ranger Russet event F10 had significantly less disease severity than the Ranger Russet control. With the exception of Ranger Russet event F10, no differences were observed between the events and the untransformed controls.

Tuber Susceptibility to Soft Rot

In 2009, tubers were tested for susceptibility to *Erwinia carotovora* subsp. *carotovora*, causal agent of bacterial soft rot, under laboratory conditions. For the assay represented in **Table 5**, two repetitions of ten field-grown tubers per event were inoculated with a suspension of Erwinia in water (OD_{600} = 0.6). These tubers were grown in Canyon County, ID, for Ranger Russet and Russet Burbank controls and events, and Oneida County, WI, for G and H controls and events.

Table 5. Weight Loss (%) in *Erwinia*-infected Tubers[1]

Line	Mean Weight Loss ± Std. Error[2]
Ranger Control	0.709 ± 0.204
F37	0.654 ± 0.177
Burbank Control	1.474 ± 0.589
E12	1.161 ± 0.200
E24	1.493 ± 0.768
G Control	8.8 ± 4.5
G11	6.0 ± 1.6
H Control	3.2 ± 1.0
H37	2.8 ± 0.6

[1]Data in this table are from tubers harvested in 2009.

[2]Based on a comparison of means and their associated Std. Errors, no differences were observed between any event and its untransformed counterpart.

The results of the tuber assay used to determine susceptibility to the bacterial soft rot pathogen, *Erwinia carotovora* subsp. *carotovora* of tubers harvested in 2009 are presented in **Table 5**. These data indicate that the percent weight loss of untransformed controls did not differ from the events. Thus, independent lines of two chipping varieties and two French fry varieties with low *Ppo* expression in tubers were shown to have similar susceptibility to bacterial soft rot to the corresponding untransformed control for each variety. This supports the expectation that *Ppo5* gene silencing in mature tubers would not cause altered susceptibility to bacterial soft rot.

Additional tests were conducted with potatoes harvested in 2011. For the assay represented in **Table 6**, tubers of Ranger Russet, Russet Burbank and Atlantic controls and events were grown at two sites, Adams County, WA and Canyon County, ID. Tubers of H variety control and events were from two separate sites in Oneida County, WI, and G control and event were from one site in Oneida County, WI. Three repetitions of ten field-grown tubers per line were assessed for susceptibility to soft rot, with the exception of the Atlantic control and events from Canyon County, ID, for which three repetitions of eight field-grown tubers were tested.

Table 6. Weight Loss (%) in *Erwinia*-infected Tubers*

Line	Mean Weight Loss (%)	Range (%)	P-value[1]
Ranger Control	1.80	1.19-2.40	
F10	1.81	1.37-2.24	0.9604
F37	1.92	1.64-2.41	0.4956
Burbank Control	2.04	1.73-2.61	
E12	1.57	1.19-1.86	0.1520
E24	1.83	1.44-2.62	0.4553
Atlantic Control	3.30	2.39-4.67	
J3	2.73	2.33-3.21	0.0153
J55	2.67	2.24-3.43	0.0085
J78	3.11	2.82-3.38	0.3665
G Control	4.17	3.77-4.71	
G11	3.98	3.17-4.62	0.4679
H Control	4.16	3.68-4.58	
H37	3.44	2.46-5.52	0.0694
H50	3.40	2.8-4.07	0.0600

*Data in this table are for tubers harvested in 2011.

[1]P-values determined using Tukey-Kramer HSD with a significance level of $P < 0.05$. P-values indicating significant differences with controls are underlined.

The results of the tuber assay used to determine susceptibility to the bacterial soft rot pathogen, *Erwinia carotovora* subsp. *carotovora* of tubers harvested in 2011 are presented in **Table 6**. These data show that the percent weight loss of untransformed controls did not differ significantly from the events, except in the case of the Atlantic variety control compared to the J3 and J55 events. Events J3 and J55 displayed a lowered susceptibility to bacterial soft rot than both the untransformed Atlantic control and event J78. These data support the claim that *Ppo5* gene silencing in mature tubers is not linked to altered susceptibility to bacterial soft rot in these potato events.

MATERIALS and METHODS

Mycelial Inoculation for Late Blight in Tubers.
The method used here is based on previously developed methods by Kirk et al. (1999). In another paper, Lambert et al. (1998) used a mycelium-based inoculation method to determine infection efficiency. The method was used more recently by Gigot et al. (2009) in a paper studying colonization and sporulation of *Phytophthora infestans* on volunteer potatoes under Western Washington conditions. There are several reasons for using mycelium based innoculations: 1) after artificial inoculation with zoospores/sporangia the spores develop mycelium, 2) tubers received for testing have well formed periderm reducing the potential efficacy of a surface inoculation, 3) well formed periderm usually has to be disrupted to enable efficient infection, 4) introduction of a zoospore/sporangial suspension by needle is also an unnatural infection process, and 5) the plug-method has produced consistent results over 5 years of testing. Addtitionally, the advantages of these methods are: 1) low risk of losing inoculum from leakage if tubers are disturbed immediately after inoculation, 2) location can be precise (1 cm distal from the dominant sprout) as with zoospore/sporangial method, 3) the progress of disease development can be followed through the tuber from apical to basal regions of the tuber, 4) and after inoculation the disease progress through the tuber is consistent across samples (Personal Communication with Dr. Willie Kirk, Michigan State University).

Late blight assay for tubers harvested in 2009. Tubers were surface sterilized in a 5% bleach solution, rinsed and dried overnight prior to inoculation. Ranger Russet and Russet Burbank tubers were inoculated by removing a 5 mm diameter plug (10 mm depth) at the apical end about 1 cm from the dominant sprout, and placing a mycelia agar plug (mycelia down) in the hole, and covering the tuber plug with a dab of petroleum jelly. Each sub-peridermal inoculation contained about 2×10^{-5} sporangia per plug. The non-inoculated control tubers were inoculated with an agar plug."

The infected tubers were incubated at 95% relative humidity and 10^{o}C and scored, on a 0-9 scale, for disease symptoms 2 months after inoculation. A zero score meant there were no visible late blight disease symptoms; that is, no tuber discoloration, no surface mycelium and no physical tissue breakdown. A score of 9 was indicative of 100% late blight infection manifested by mycelium on the entire tuber surface and either with or without tissue discoloration and breakdown.

Susceptibility to late blight disease was assessed digitally in the G and H lines. Digital scans were taken of tuber slices from the apical, middle and basal portions of the inoculated tubers and diseased area was quantitated using image analysis software (SigmaScan, Jandel Scientific Software, San Rafael, CA). The average reflective intensity of light reflected from the cut surface measures the darkened, diseased tuber tissue amid the lighter, symptom-free tissue.

G and H tubers were inoculated on the apical end with the US8, US10, and US22 genotypes, and scored by digitally scanning the apical, middle, and basal sections of the tuber. The diseased area was used to calculate a Relative Average Reflective Index (RARI) (Niemira *et al*. 1999) with image analysis software (SigmaScan, Jandel Scientific Software, San Rafael, Ca). A lower RARI value indicates less disease. RARI values across the apical, middle, and basal sections were combined.

Late blight assay for tubers harvested in 2011. 4 tubers each for events and controls were surface sterilized prior to inoculation. The apical ends of the tubers were inoculated by removing a plug of tissue with a No. 4 cork borer, placing a *P. infestans* mycelia plug (mycelia down), replacing the tuber core and sealing with a dab of petroleum jelly. The infected tubers were placed in plastic crates and incubated for

58 days at 10^{o}C within large plastic bags to maintain high humidity. Upon analysis, tubers were halved longitudinally from apical to basal end, and evaluated for percent late blight infection of the tuber. Tubers were inoculated with both *P. infestans* strains US8 and US22. All tubers infected with the US22 genotype did not display late blight symptoms, which is most likely due to a lack of infection, as opposed to higher tuber resistance. The data for tubers unsuccessfully infected with US22 are not presented here. However, typical disease progression was observed in tubers infected with US8, and those results are reported within this document.

Late blight foliar assay for plants grown in 2011. The late blight field trial was planted in Michigan on 6/9/2011 as a RCBD consisting of 3 row blocks of 5-hill plots with 3 replications. The trial was inoculated on 8/1/2011 with a Michigan isolate of *P. infestans* genotype US22. Disease ratings were taken at eight different dates over a 54 DAI (Days After Inoculation) period. Plot percent late blight disease rating was used to calculate the Relative Area Under the Disease Progression Curve (RAUDPC x100) which was used to evaluate disease severity.

Erwinia **assay for tubers harvested in 2009.** Each tuber was wounded three times using a sterile, disposable pipette tip. Each wound was made to a depth of 15 mm around a central point between the stem and stolon end. 50 µl of *Erwinia* suspension was pipetted onto each wound. Inoculated tubers were incubated at 22 ^{o}C and 95% relative humidity. After 7 days of incubation for the smaller tubers of the chipping varieties (G and H) and 9 days incubation for the more tolerant frying varieties (Ranger Russet, Russet Burbank), tubers were scored for susceptibility to soft rot. The fresh weight of each tuber was recorded. Tubers were then cut into two or three pieces to expose the rotted tissue inside. With a high pressure stream of water, rotted tissue was forcefully removed from healthy tissue. The tubers were air dried and reweighed. The percentage fresh weight loss due to rot was recorded.

Erwinia **assay for tubers harvested in 2011.** The fresh weight of each tuber was recorded. Tubers were surface sterilized with 10% bleach solution for 10 minutes, rinsed thoroughly with water, and allowed to air dry. Each tuber was wounded by removing a 2 cm long plug using a No. 4 cork borer, and removing a 2 mm piece of the plug from the internal end to allow room for the *Erwinia* culture. 100 µl of *Erwinia* suspension in Luria broth was pipetted into the wound, and the plug replaced and sealed with petroleum jelly. Inoculated tubers were incubated at 20^{o}C and 95% relative humidity. After 7 days of incubation, tubers were scored for susceptibility to soft rot. Tubers were cut in half to expose the rotted tissue inside. With a spatula, rotted tissue was removed from healthy tissue. The tubers were then reweighed and the percentage fresh weight loss due to rot was recorded.

CONCLUSIONS

It can be concluded that potato transformation with the DNA insert of pSIM1278 does not alter disease susceptibility and, consequently, does not trigger environmental safety issues associated with the spread of plant diseases. All 10 events were included in tests for *Phytophtora* and *Erwinia*, although the G and H events were not part of the *Phytophtora* foliar and tuber testing conducted in 2011. Some differences were observed in the susceptibility to *P. infestans* of tubers of events E12 and J3, and foliage of F10. All events from the 2011 harvest were tested for *Erwinia* soft rot, and the only differences were slightly less susceptibility in Atlantic events J3 and J55. Considering that the 10 events transformed with pSIM1278 do not show any clear trends for altered susceptibility to these disease agents, it is expected that G and H events would have performed similarly in additional tests. When *Phytophtora* was studied in both foliage and tubers (Porter et al. 2004), those varieties that were most resistant in foliage also showed resistance in tubers. Conclusions related to each individual event are summarized below.

Event Ranger Russet F10: Tubers of this event showed similar susceptibility to *P. infestans* or *Erwinia* compared with tubers of untransformed Ranger Russet. Foliage of this event showed less susceptibility to *P. infestans* compared with the untransformed Ranger Russet.

Event Ranger Russet F37: Tubers of event F37 showed similar susceptibility to *P. infestans* or *Erwinia* compared with untransformed Ranger Russet. Foliage of this event was not tested.

Event Russet Burbank E12: Tubers of event E12 displayed more susceptibility to *P. infestans*, but not *Erwinia* when compared with tubers of untransformed Russet Burbank harvested in 2009. Although we observed more susceptibility in the 2009 study, when the assay was repeated with tubers harvested in 2011, tubers of E12 were found to display lower late blight symptoms than the Russet Burbank control. Foliage of this event showed similar susceptibility to *P. infestans* compared with the untransformed Russet Burbank.

Event Russet Burbank E24: Tubers of event E24 showed similar susceptibility to *P. infestans* or *Erwinia* compared with untransformed Russet Burbank. Foliage of this event showed similar susceptibility to *P. infestans* compared with the untransformed Russet Burbank.

Event Atlantic J3: Tubers of event J3 were more susceptible to *P. infestans* than untransformed Atlantic. However, tubers of this event showed less susceptibility to *Erwinia* than the Atlantic control. Foliage of this event showed similar susceptibility to *P. infestans* compared with the untransformed Atlantic.

Event Atlantic J55: Tubers of event J55 showed similar susceptibility to *P. infestans* compared with untransformed Atlantic. However, tubers of this event showed less susceptibility to *Erwinia* than the Atlantic control. Foliage of this event showed similar susceptibility to *P. infestans* compared with untransformed Atlantic.

Event Atlantic J78: Tubers of event J78 showed similar susceptibility to *P. infestans* or *Erwinia* compared with untransformed Atlantic. Foliage of this event showed similar susceptibility to *P. infestans* compared with untransformed Atlantic.

Event G11: Tubers of event G11 were generally less susceptible to *P. infestans,* but were no different in susceptibility to *Erwinia* than the untransformed G variety.

Event H37: Tubers of event H37 showed varied susceptibility to *P. infestans* depending, upon the strain tested when compared with the untransformed H variety. Similar susceptibility to *Erwinia* was observed between event H37 and H control.

Event H50: Tubers of event H50 showed more susceptibility to *P. infestans* strains US8 and US22, but not US10, when compared with the untransformed H variety. Similar susceptibility to *Erwinia* was observed between event H50 and H control.

REFERENCES

Gevens AJ, Seidl, AC, Clark R (2011) Potato and tomato late blight in Wisconsin: A new type of late blight in a new decade. Proc. Of the 2011 Wisconsin Crop Management Conference. 50:123-126.

Gigot, J., Gundersen, B., Inglis, D., 2009. Colonization and Sporulation of Phytophthora infestans on Volunteer Potatoes Under Western Washington Conditions. Am J Potato Res 86, 1-14.

Hakimi SM, Krohn BM, Stark DM: Monsanto Technology LLC (2006) United States Patent: Method of imparting disease resistance to plants by reducing polyphenol oxidase activities. Patent No.: US 7,122,719 B2.

Kirk, WW, Niemira, BA, Stein, JM, Hammerschmidt, R (1999) Late blight (Phytophthora infestans (Mont) de Bary) development from potato seed-pieces treated with fungicides. Pestic. Sci. 55: 1151-1158.

Lambert, D, Currier, A, Olanya, M (1998) Transmission of Phytophthora infestans in cut potato seed. Amer J of Potato Res 75: 257-263.

Li L, Steffens JC (2002) Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. Planta, 215: 239-247.

Niemira BA, Kirk WW, Stein JM (1999) Screening for late blight susceptibility in potato tubers by digital analysis of cut tuber surfaces. Plant Dis, 83: 469-473.

Porter LD, Inglis DA, Johnson DA (2004) Identification and characterization of resistance to *Phytophthora infestans* in leaves, stems, flowers, and tubers of potato clones in the Pacific Northwest. The Am Phytopath Soc, 88: 965-972.

Valentines MC, Vilaplana R, Torres R, Usall J, Larrigaudiere C (2005) Specific roles of enzymatic browning and lignifications in apple disease resistance. Postharvest Biotech and Tech, 36: 227-234.

Young GK, Cooke LR, Kirk WW, Tumbalam P, Perez FM, Deahl KL (2009) Influence of competition and host plant resistance on selection in *Phytophthora infestans* populations in Michigan, USA and in Northern Ireland. Plant Pathology, 58:703-714.

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.

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Appendix 9

Compositional Analyses

INDEX

INTRODUCTION

The field-grown tubers of both events and untransformed control varieties were analyzed to determine their compositional equivalence. Significant differences in the amount of a compound between an event and control were considered important only if the mean values were outside the normal range. In order to determine if values fell within the normal range, mean values were compared with tolerance intervals that were calculated from commercial control varieties and with values found in the scientific literature.

RESULTS

Compositional Analysis of Ranger Russet Events and Controls

Data on the biochemical composition of Ranger Russet events and controls, which represent the mean, range, p-values and/or adjusted p-values, are shown in **Tables 1** (key proximates, vitamins, and minerals), **2** (free amino acids), **3** (total amino acids), **4** (glycoalkaloids), **5** (sugars), and **6** (acrylamide in fried product).

Table 1: Ranger Russet Proximates, Vitamins, and Minerals[1]

Compound					TI	CLR[2]
		Control Ranger N=33	F10 N=33	F37 N=30		
Protein (%)	Mean	2.384	2.382	2.429	1.258-3.594	0.70-4.60
	Range	1.67-3.22	1.78-3.52	1.68-3.30		
	APV		0.4416	0.8202		
Fat (%)	Mean	0.1224	0.1263	0.1341	0.000-0.341	0.02-0.20
	Range	0.03-0.30	0.08-0.30	0.06-0.30		
	APV		0.4667	0.7585		
Ash (%)	Mean	1.103	1.140	1.115	0.391-1.888	0.44-1.90
	Range	0.14-1.54	0.00-1.67	0.33-1.52		
	APV		0.9861	1.0000		
Crude Fiber (%)	Mean	0.5282	0.5361	0.5483	0.142-0.690	0.17-3.50
	Range	0.27-0.74	0.33-0.76	0.40-0.73		
	APV		0.6080	0.7904		
Carbohydrates (%)	Mean	20.24	20.75	21.00	12.29-25.92	13.30-30.53
	Range	14.80-23.80	16.83-24.00	16.94-25.60		
	APV		0.0812	0.2112		
Calories (kcal/ 100g)	Mean	91.41	93.48	94.76	59.09-114.9	70.00-110.2
	Range	67.4-104.0	76.80-104.3	80.40-111.0		
	APV		0.0637	0.2070		
Moisture (%)	Mean	76.17	75.63	75.34	70.28-83.91	63.20-86.90
	Range	74.00-81.70	72.55-79.40	71.90-78.73		
	APV		0.0632	0.2740		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper. Adjusted P-values indicating significant differences with controls are underlined.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992), Talburt and Smith (1987).

Table 1 (Continued): Ranger Russet Proximates, Vitamins, and Minerals[1]

Compound					TI	CLR[2]
		Control Ranger N=33	F10 N=33	F37 N=30		
B_3 (mg/ 100g)	Mean	2.415	2.500	2.698	0.922-3.123	0.09-3.10
	Range	1.43-3.43	1.90-3.43	2.00-3.70		
	APV		0.0006	0.0077		
B_6 (mg/ 100g)	Mean	0.1348	0.1352	0.1299	0.059-0.192	0.13-0.41
	Range	0.11-0.16	0.10-0.22	0.10-0.18		
	APV		0.2953	0.6397		
Vitamin C (mg/ 100g)	Mean	25.87	30.68	29.95	0.000-129.3	1.00-54.00
	Range	11.36-51.40	16.16-56.50	16.15-59.00		
	APV		0.0039	0.0254		
Cu (ppm)	Mean	1.1637	1.1210	1.1182	0.111-2.236	0.15-7.00
	Range	0.75-1.85	0.64-1.61	0.73-1.60		
	APV		0.4992	0.7211		
Mg (ppm)	Mean	284.8	285.7	287.8	101.5-371.5	112.5-550.0
	Range	204.6-405.6	207.6-408.2	204.4-420.1		
	APV		0.6687	0.7903		
K (ppm)	Mean	4982	5088	5069	2711-6882	3500.-6250
	Range	3670-6885	3900-7432	3770-7049		
	APV		0.9992	0.9992		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper, ppm = parts per million. Adjusted P-values indicating significant differences with controls are underlined.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992), Talburt and Smith (1987).

Table 2: Ranger Russet Free Amino Acids (parts per million)[1]

Free Amino Acid (ppm)		Control Ranger N=32	F10 N=33	F37 N=33	TI[2]	CLR[3]
ALA	Mean	108.9	134.4	154.8	100.0-208.2	12.00-236.0
	Range	100.0-182.0	100.0-248.0	100.0-305.0		
	APV		NA	NA		
ARG	Mean	707.3	948.0	1011	100.0-1068	120.0-1472
	Range	352.0-1218	332.5-1740	381.0-1885		
	APV		0.1692	0.0470		
ASN	Mean	2351	567.2	642.5	500.0-4952	312.0-6980
	Range	1209-3530	381.8-872.0	355.0-1100		
	APV		0.0266	0.0206		
ASP	Mean	558.5	600.2	624.9	100.0-1411	64.00-752.0
	Range	266.0-1197	242.0-1225	303.0-1279		
	APV		0.1490	0.0077		
GLN	Mean	958.6	1668	1715	100.0-3449	440.0-5396
	Range	399.1-1648	409.0-2860	718.0-2610		
	APV		<0.0001	<0.0001		
GLU	Mean	557.4	652.4	657.0	100.0-1192	450.0-742.0
	Range	252.7-877.9	302.9-883.4	301.5-966.0		
	APV		0.0896	0.0632		
GLY	Mean	100.0	100.5	100.1	100.0-116.1	2.00-104.0
	Range	100.0-100.0	100.0-114.4	100.0-103.4		
	APV		NA	NA		
HIS	Mean	101.1	101.8	103.1	100.0-136.9	34.00-656.0
	Range	100.0-126.0	100.0-125.0	100.0-138.0		
	APV		NA	NA		
ILE	Mean	129.4	120.3	142.0	100.0-217.8	28.00-330.0
	Range	100.0-223.0	100.0-172.4	100.0-226.8		
	APV		NA	NA		
LEU	Mean	100.7	100.7	100.2	100.0-173.9	16.00-262.0
	Range	100.0-120.9	100.0-123.8	100.0-104.0		
	APV		NA	NA		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3]CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010). GLN high level reported as 5396 ppm from the mean of 4 sites (Davies 1977) omitting a level of 18,244 ppm from a single site, which appeared to be an outlier.

Table 2 (Continued): Ranger Russet Free Amino Acids (parts per million)[1]

Free Amino Acid (ppm)					TI[2]	CLR[3]
		Control Ranger N=32	F10 N=33	F37 N=33		
LYS	Mean	175.3	180.1	195.3	100.0-557.3	18.00-638.0
	Range	100.0-435.3	100.0-372.8	100.0-400.1		
	APV		0.8186	0.2167		
MET	Mean	114.5	114.5	118.9	100.0-191.6	18.00-216.0
	Range	100.0-222.0	100.0-178.0	100.0-208.0		
	APV		NA	NA		
PHE	Mean	168.6	158.2	174.1	100.0-294.9	0.00-408.0
	Range	100.0-248.0	100.0-294.7	100.0-250.0		
	APV		0.5366	0.7848		
PRO	Mean	169.8	204.8	277.3	100.0-630.5	0.00-968.0
	Range	100.0-458.6	100.0-747.8	100.0-762.0		
	APV		NA	NA		
SER	Mean	113.4	122.9	137.7	100.0-198.1	30.00-256.0
	Range	100.0-178.0	100.0-218.0	100.0-237.0		
	APV		NA	NA		
THR	Mean	120.2	135.5	144.8	100.0-219.8	28.00-286.0
	Range	100.0-186.0	100.0-244.7	100.0-239.1		
	APV		NA	NA		
TRP	Mean	100.0	100.0	100.0	100.0-113.1	14.00-348.0
	Range	100.0-100.0	100.0-100.0	100.0-100.0		
	APV		NA	NA		
TYR	Mean	103.1	110.6	115.2	100.0-225.2	18.00-638.0
	Range	100.0-140.0	100.0-198.0	100.0-188.0		
	APV		0.5223	0.8052		
VAL	Mean	236.1	236.4	281.3	100.0-460.6	30.00-740.0
	Range	125.6-476.0	117.5-416.0	100.0-497.0		
	APV		0.9486	0.0681		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010).

Table 3: Ranger Russet Total Amino Acids (parts per milllion)[1]

Total Amino Acid (ppm)		Control Ranger N=32	F10 N=33	F37 N=33	TI	CLR[2]
ALA	Mean	661.3	715.1	729.3	100.0-1335	392.0-952.0
	Range	448.3-1090	389.8-1179	473.3-956.3		
	APV		0.5063	0.4379		
ARG	Mean	1174	1439	1495	367.6-1922	700.0-1383
	Range	765.4-2344	711.0-2297	955.9-2767		
	APV		0.1291	0.0719		
ASP+ASN	Mean	5518	3044	3121	100.0-13340	3385-7380
	Range	2080-8486	1960-5572	2010-4571		
	APV		0.0165	0.0182		
GLU+GLN	Mean	3144	4368	4380	568.4-7011	2915-6035
	Range	2378-4223	2600-6070	2760-5690		
	APV		<0.0001	<0.0001		
GLY	Mean	1468	1607	1472	100.0-2865	500.0-1990
	Range	462.0-3088	484.0-3284	463.0-3457		
	APV		0.7382	0.9446		
HIS	Mean	424.0	420.3	466.5	100.0-760.7	133.0-469.0
	Range	100.0-887.8	208.9-851.5	145.2-1034		
	APV		0.9038	0.1934		
ILE	Mean	717.5	771.7	755.7	144.8-1346	525.0-953.0
	Range	380.0-961.2	431.8-1186	507.0-1016		
	APV		0.3851	0.5198		
LEU	Mean	1386	1460	1455	476.9-2174	685.0-1383
	Range	911.0-2103	939.0-2119	920.0-2225		
	APV		0.4719	0.4808		
LYS	Mean	992.4	1137	1125	100.0-3363	687.0-1368
	Range	745.5-1177	809.4-1808	868.0-1616		
	APV		0.1320	0.1763		
MET	Mean	371.0	395.2	402.2	100.0-593.3	300.0-500.0
	Range	140.2-836.4	218.5-744.7	214.6-682.1		
	APV		0.2895	0.2189		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 3 (Continued): Ranger Russet Total Amino Acids (parts per milllion)[1]

Total Amino Acid (ppm)		Control Ranger N=32	F10 N=33	F37 N=33	TI	CLR[2]
PHE	Mean	857.9	850.3	874.3	397.1-1395	552.0-1087
	Range	597.7-1208	646.0-1262	637.6-1589		
	APV		0.8401	0.6817		
PRO	Mean	809.4	886.1	919.9	100.0-1492	355.0-1464
	Range	564.0-1473	567.7-1747	574.0-1861		
	APV		0.4608	0.2788		
SER	Mean	669.0	727.5	693.4	100.0-1362	500.0-1022
	Range	318.7-1403	293.8-1205	345.3-1542		
	APV		0.0996	0.5270		
THR	Mean	682.5	755.5	739.6	226.0-1315	436.0-855.0
	Range	279.4-1070	379.0-1465	445.4-1287		
	APV		0.0848	0.1662		
TRP	Mean	216.8	217.9	214.3	114.7-434.7	14.00-348.0
	Range	192.0-243.0	174.0-272.0	179.0-261.0		
	APV		0.8646	0.7703		
TYR	Mean	590.5	642.4	649.9	100.0-1523	457.0-942.0
	Range	317.3-760.0	469.3-933.0	458.5-915.0		
	APV		0.0887	0.0542		
VAL	Mean	1007	1031	1014	100.0-1860	752.0-1450
	Range	559.9-1512	656.2-1657	639.4-1319		
	APV		0.8817	0.9906		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 4: Ranger Russet Glycoalkaloids (mg/100 g)[1]

Glycoalkaloids (mg/100g)				TI	CLR[2]
	Control Ranger N=32	F10 N=33	F37 N=33		
Mean	6.704	6.979	6.805	0.000-33.05	3.20 – 210.4
Range	3.06-10.23	2.84-9.51	4.28-9.42		
P-value		0.8285	0.9812		

[1]TI = Tolerance Interval, CLR = Combined Literature Range , P-values indicating significant differences with controls are underlined.
[2]Literature ranges from Kozukue *et al.* (2008).

Table 5: Ranger Russet Sugars (percentage fresh weight)[1]

Fructose + Glucose (%)						
		Ranger Control	F10	F37	TI[1]	CLR[2]
Fructose + Glucose Fresh	Mean	0.185	0.182	0.178	0.000-0.307	0.00-0.80
	Range	0.047-0.362	0.040-0.437	0.034-0.374		
	P-Value		0.9924	0.9613		
	N	12	12	12		
Fructose + Glucose Month 1	Mean	0.086	0.069	0.057	0.000-0.307	0.00-0.80
	Range	0.043-0.141	0.036-0.110	0.038-0.075		
	P-Value		0.0243	0.0009		
	N	21	21	17		
Fructose + Glucose Month 3	Mean	0.270	0.245	0.286	0.000-0.307	0.00-0.80
	Range	0.073-0.450	0.068-0.550	0.082-0.552		
	P-Value		0.6579	0.8986		
	N	27	27	24		
Fructose + Glucose Month 5	Mean	0.190	0.178	0.224	0.000-0.307	0.00-0.80
	Range	0.083-0.412	0.073-0.358	0.069-0.602		
	P-Value		0.9696	0.9571		
	N	21	21	18		
Sucrose (%)						
		Ranger Control	F10	F37	TI	CLR
Sucrose Fresh	Mean	0.188	0.174	0.188	0.000-0.315	0.05-0.32
	Range	0.082-0.293	0.080-0.252	0.043-0.284		
	P-Value		0.7892	0.9999		
	N	12	12	12		
Sucrose Month 1	Mean	0.137	0.136	0.138	0.000-0.315	0.05-0.32
	Range	0.094-0.179	0.073-0.186	0.077-0.183		
	P-Value		0.9327	0.9826		
	N	21	21	17		
Sucrose Month 3	Mean	0.061	0.063	0.056	0.000-0.315	0.05-0.32
	Range	0.009-0.127	0.018-0.121	0.014-0.122		
	P-Value		0.7420	0.9032		
	N	27	27	24		
Sucrose Month 5	Mean	0.068	0.067	0.055	0.000-0.315	0.05-0.32
	Range	0.008-0.129	0.009-0.135	0.008-0.150		
	P-Value		0.9367	0.7556		
	N	21	21	18		

[1]TI = Tolerance Interval, CLR = Combined Literature Range , P-values indicating significant differences with controls are underlined.

[2]Literature ranges from Blenkinsop *et al.* (2002); Matsuura-Endo *et al.* (2006); Menendez *et al.* (2002).

Table 6: Ranger Russet, Acrylamide Levels in Processed Material (parts per billion)[1]

Acrylamide (ppb)		Ranger Control	F10	F37
Fresh 2010 & 2011	Mean	371.9	150.6	127.3
	Range	175-513	98-236	62-242
	P-value	—	<0.0001	<0.0001
	N	36	36	36
Month 2 2009	Mean	588.4	207.7	210.3
	Range	436-688	135-254	164-254
	P-value	—	<0.0001	<0.0001
	N	9	9	6
Month 3 2010 & 2011	Mean	630.6	237.9	221.8
	Range	366-1010	132-370	120-374
	P-value	—	<0.0001	<0.0001
	N	23	24	24
Month 5 2009	Mean	148.0	70.8	81.7
	Range	124-196	54-83	78-88
	P-value	—	<0.0001	<0.0001
	N	9	9	6
Month 6 2010	Mean	460.4	144.3	180.8
	Range	171-714	77-195	112-299
	P-value	—	<0.0001	<0.0001
	N	12	12	12
Month 7 2009	Mean	443.0	145.7	161.3
	Range	359-497	130-173	146-190
	P-value	—	<0.0001	<0.0001
	N	9	9	6

[1]P-values indicating significant differences with controls are underlined.

Compositional Analysis of Event F10

Tubers of event F10 were equivalent to those of untransformed Ranger Russet except for the following *expected* changes:

- F10 tubers contained, on average, 567 parts per million (ppm) free ASN as compared to controls, which contained 2351 ppm (APV = 0.0266). This change was expected and is linked to a reduced acrylamide-forming potential.

- F10 tubers contained, on average, 1668 ppm free GLN as compared to controls, which contained 959 ppm (APV = <0.0001). The increase in this amide amino acid counter-balances, in part, the losses in the other amide amino acid ASN. The new levels are still normal (which means, within the normal range) for potato.

- F10 tubers contained, on average, 3044 ppm total ASN+ASP as compared to controls, which contained 5518 ppm (APV = 0.0165). The new level fits within the tolerance interval and is still normal for potato.

- F10 tubers contained, on average, 4368 ppm total GLN+GLU as compared to controls, which contained 3144 ppm (APV <0.0001). The new levels are still normal for potato.

- F10 tubers at 1 month after harvest contained, on average, 0.069% of the reducing sugars glucose plus fructose as compared to controls, which contained 0.086% (P-value = 0.0243). The decrease in reducing sugars was expected from silencing the promoters associated with the *R1* and *PhL* genes. The new levels are still normal for potato.

- French fries from potato strips from fresh F10 potato tubers contained 150.6 ppb acrylamide as compared to the control, which had 371.9 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in free ASN, free GLN, total ASN + ASP, and total GLN + GLU because as non-essential amino acids they can be synthesized by the body. The observed decrease in reducing sugars falls within the normal range for potatoes. However, the combination of lower ASN and reducing sugars contribute to the reduced potential of F10 to form acrylamide and would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

The following changes were *observed*:

- F10 tubers contained, on average, 2.5 mg/100 g vitamin B3 as compared to control, which contained 2.4 mg/100 g (APV = 0.0006). The new levels are still normal for potato.

- F10 tubers contained, on average, 30.7 mg/100 g vitamin C as compared to control, which contained 25.9 mg/100 g (APV = 0.0039). The new levels are still normal for potato.

These changes result in a very small modification in the vitamin composition of Ranger Russet F10 that is still standard for potato and will not significantly alter potential dietary intake levels. In addition, the levels were within the normal range for potatoes.

It can be concluded that tubers of F10 are substantially equivalent to untransformed Ranger Russet controls except for: (1) lowered free ASN, (2) higher levels of free GLN, (3) lower levels of total ASP + ASN, (4) higher levels of total GLN + GLU, (5) the expected decreased levels of the reducing sugars glucose plus fructose, (6) reduced acrylamide as expected and intended, and (7) slightly increased levels of vitamin B3 and vitamin C. In all cases, these compositional changes fall within the normal range for potatoes.

Compositional Analysis of Event F37

Tubers of event F37 were equivalent to those of untransformed Ranger Russet except for the following expected changes:

- F37 tubers contained, on average, 643 parts per million (ppm) free ASN as compared to controls, which contained 2351 ppm (APV = 0.0206). This change was expected and is linked to a reduced acrylamide-forming potential.

- F37 tubers contained, on average, 625 parts per million (ppm) free ASP as compared to controls, which contained 559 ppm (APV = 0.0077). These levels were within the normal range for potatoes.

- F37 tubers contained, on average, 1715 ppm free GLN as compared to controls, which contained 959 ppm (APV <0.0001). The increase in this amide amino acid counter-balances, in part, the losses in the other amide amino acid ASN. The new levels are still normal for potato.

- F37 tubers contained, on average, 3121 ppm total ASN + ASP as compared to controls, which contained 5518 ppm (APV = 0.0182). The new level fits within the tolerance interval and is still normal for potato.

- F37 tubers contained, on average, 4380 ppm total GLN + GLU as compared to controls, which contained 3144 ppm (APV <0.0001). The new levels are still normal for potato.

- F37 tubers at 1 month after harvest contained, on average, 0.057% of the reducing sugars glucose plus fructose as compared to controls, which contained 0.086% (P-value = 0.0009). The decrease in reducing sugars was expected from silencing the promoters associated with the *R1* and *PhL* genes. The new levels are still normal for potato.

- French fries from potato strips from fresh F37 potato tubers contained 127.3 ppb acrylamide as compared to the control, which had 371.9 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in free ASN, ASP, GLN, total ASN + ASP, and total GLN + GLU because as non-essential amino acids they can be synthesized by the body. The observed decrease in reducing sugars falls within the normal range for potatoes. However, lower ASN and reducing sugars contributes to the reduced potential of F37 to form acrylamide and would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

The following changes were *observed*:

- F37 tubers contained, on average, 1011 parts per million (ppm) free ARG as compared to controls, which contained 707 ppm (APV = 0.0470). These levels were within the normal range for potatoes.

- F37 tubers contained, on average, 29.9 mg/100 g vitamin C as compared to controls, which contained 25.9 mg/100 g (APV = 0.0254). The new levels are still normal for potato.
- F37 tubers contained, on average, 2.70 mg/100 g niacin (vitamin B_3) as compared to controls, which contained 2.42 mg/100 g (APV = 0.0077). The new levels are still normal for potato.

These changes result in very small modifications in the vitamin composition of Ranger Russet F37 that are still standard for potato and will not significantly alter potential dietary intake levels.

It can be concluded that tubers of F37 are substantially equivalent to untransformed Ranger Russet controls except for: (1) lowered free ASN, (2) higher levels of free ASP and free GLN, (3) lower levels of total ASP + ASN, (4) higher levels of total GLU + GLN, (5) decreased levels of the reducing sugars glucose plus fructose, (6) reduced acrylamide as expected and intended, (7) slightly increased levels of vitamin C and niacin (vitamin B_3), and (8) increased levels of free ARG. In all cases, these compositional changes fall within the normal range for potatoes.

Compositional Analysis of Russet Burbank Events and Controls

Data on the biochemical composition of Russet Burbank events and controls, which represent the mean, range, p-values and/or adjusted p-values, are shown in **Tables 7** (key proximates, vitamins, and minerals), **8** (free amino acids), **9** (total amino acids), **10** (glycoalkaloids), **11** (sugars), and **12** (acrylamide in fried product).

Table 7: Russet Burbank Proximates, Vitamins, and Minerals[1]

Compound					TI	CLR[2]
		Control Burbank N=33	E12 N=33	E24 N=33		
Protein (%)	Mean	2.317	2.298	2.289	1.258-3.594	0.70-4.60
	Range	1.64-3.10	1.47-3.02	1.56-3.04		
	APV		1.0000	0.7732		
Fat (%)	Mean	0.100	0.101	0.108	0.000-0.341	0.02-0.20
	Range	0.03-0.20	0.05-0.20	0.05-0.20		
	APV		NA	NA		
Ash (%)	Mean	1.079	1.030	1.052	0.391-1.888	0.44-1.90
	Range	0.15-2.00	0.00-1.46	0.56-1.42		
	APV		1.0000	0.8379		
Crude Fiber (%)	Mean	0.470	0.467	0.456	0.142-0.690	0.17-3.50
	Range	0.32-0.64	0.33-0.70	0.32-0.69		
	APV		1.0000	1.0000		
Carbohydrates (%)	Mean	18.49	18.50	18.27	12.29-25.92	13.30-30.53
	Range	16.10-21.10	14.20-22.20	14.80-22.00		
	APV		0.9806	0.7676		
Calories (kcal/100g)	Mean	84.00	83.90	82.98	59.09-114.9	70.00-110.2
	Range	73.10-93.26	64.90-96.30	68.40-95.80		
	APV		1.0000	0.7774		
Moisture (%)	Mean	78.02	78.09	78.31	70.28-83.91	63.20-86.90
	Range	75.51-80.40	75.50-82.50	75.04-81.70		
	APV		1.0000	0.7775		
B3 (mg/100g)	Mean	1.865	1.901	1.847	0.922-3.123	0.09-3.10
	Range	1.44-2.38	1.43-2.53	1.46-2.37		
	APV		1.0000	0.8855		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992), Talburt and Smith (1987).

Table 7 (Continued): Russet Burbank Proximates, Vitamins, and Minerals[1]

Compound					TI	CLR[2]
		Control Burbank N=33	E12 N=33	E24 N=33		
B6 (mg/ 100g)	Mean	0.143	0.142	0.144	0.059-0.192	0.13-0.41
	Range	0.11-0.20	0.11-0.23	0.10-0.22		
	APV		1.000	0.8778		
Vitamin C (mg/ 100g)	Mean	17.14	18.76	18.75	0.000-129.3	1.00-54.00
	Range	8.88-42.90	11.84-32.90	11.06-33.80		
	APV		1.0000	0.9118		
Cu (ppm)	Mean	0.965	1.002	0.918	0.111-2.236	0.15-7.00
	Range	0.50-1.34	0.54-1.52	0.56-1.50		
	APV		1.0000	1.0000		
Mg (ppm)	Mean	241.7	246.4	242.0	101.5-371.5	112.5-550.0
	Range	165.0-350.2	187.6-405.0	179.3-360.1		
	APV		1.0000	0.9658		
K (ppm)	Mean	4681	4777	4577	2711-6882	3500-6250
	Range	3040-6339	3550-6537	3280-6537		
	APV		1.0000	1.0000		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper, ppm = parts per million. Adjusted P-values indicating significant differences with controls are underlined.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992), Talburt and Smith (1987).

Table 8: Russet Burbank Free Amino Acids (parts per million)[1]

Free Amino Acid (ppm)		Control Burbank N=33	E12 N=33	E24 N=33	TI[2]	CLR[3]
ALA	Mean	156.4	189.4	157.7	100.0-208.2	12.00-236.0
	Range	100.0-332.7	100.0-354.7	100.0-298.1		
	APV		NA	NA		
ARG	Mean	519.6	525.2	484.8	100.0-1068	120.0-1472
	Range	257.1-833.2	242.5-912.9	261.9-747.0		
	APV		0.8671	0.5349		
ASN	Mean	2421	618.2	591.9	500.0-4952	312.0-6890
	Range	822.3-4030	425.2-1250	416.0-980.0		
	APV		0.1148	0.1088		
ASP	Mean	438.9	447.5	451.7	100.0-1411	64.00-752.0
	Range	280.3-760.1	293.4-823.9	280.5-743.8		
	APV		0.6551	0.5938		
GLN	Mean	1111	1997	1979	100.0-3449	440.00-5396
	Range	605.4-1700	865.2-3490	885.0-3220		
	APV		0.0767	0.0844		
GLU	Mean	465.7	459.9	453.0	100.0-1192	450.0-742.0
	Range	221.1-688.9	227.3-741.0	240.8-721.0		
	APV		0.9548	0.6847		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010). GLN high level reported as 5396 ppm from the mean of 4 sites (Davies 1977) omitting a level of 18,244 ppm from a single site, which appeared to be an outlier.

Table 8 (Continued): Russet Burbank Free Amino Acids (parts per million)[1]

Free Amino Acid (ppm)					TI[2]	CLR[3]
		Control Burbank N=33	E12 N=33	E24 N=33		
GLY	Mean	100.1	102.2	101.2	100.0-116.1	2.00-104.0
	Range	100.0-104.2	100.0-127.0	100.0-120.7		
	APV		NA	NA		
HIS	Mean	107.6	107.5	104.9	100.0-136.9	34.00-656.0
	Range	100.0-157.0	100.0-170.0	100.0-143.0		
	APV		NA	NA		
ILE	Mean	152.0	157.6	140.2	100.0-217.8	28.00-330.0
	Range	100.0-276.0	100.0-303.3	100.0-279.2		
	APV		0.8093	0.5534		
LEU	Mean	145.7	140.4	124.7	100.0-173.9	16.00-262.0
	Range	100.0-267.1	100.0-302.3	100.0-211.5		
	APV		NA	NA		
LYS	Mean	227.6	210.0	188.1	100.0-557.3	18.00-638.0
	Range	100.0-384.6	100.0-407.6	100.0-286.0		
	APV		0.7610	0.2799		
MET	Mean	159.7	174.8	161.7	100.0-191.6	18.00-216.0
	Range	105.3-233.0	103.4-251.0	116.9-226.0		
	APV		0.4689	0.8393		
PHE	Mean	213.7	196.1	173.6	100.0-294.9	0.00-408.0
	Range	100.0-359.0	100.0-354.0	100.0-283.6		
	APV		0.6358	0.1708		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010).

Table 8 (Continued): Russet Burbank Free Amino Acids (parts per million)[1]

Free Amino Acid (ppm)					TI[2]	CLR[3]
		Control Burbank N=33	E12 N=33	E24 N=33		
PRO	Mean	144.2	159.8	134.4	100.0-630.5	0.00-968.0
	Range	100.0-313.7	100.0-354.2	100.0-258.3		
	APV		NA	NA		
SER	Mean	135.9	137.0	138.0	100.0-198.1	30.00-256.0
	Range	100.0-277.0	100.0-282.0	100.0-251.0		
	APV		NA	NA		
THR	Mean	124.2	144.0	136.9	100.0-219.8	28.00-286.0
	Range	100.0-194.7	100.0-243.3	100.0-213.4		
	APV		NA	NA		
TRP	Mean	100.5	100.5	100.0	100.0-113.1	14.00-348.0
	Range	100.0-111.5	100.0-109.9	100.0-100.0		
	APV		NA	NA		
TYR	Mean	152.5	181.2	167.3	100.0-225.2	18.00-638.0
	Range	100.0-336.5	100.0-290.0	100.0-289.9		
	APV		NA	NA		
VAL	Mean	359.5	380.7	347.0	100.0-460.6	30.00-740.0
	Range	212.5-578.0	186.6-624.0	196.3-505.0		
	APV		0.3722	0.5272		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010).

Table 9: Russet Burbank Total Amino Acids (parts per million)[1]

Total Amino Acid (ppm)					TI	CLR[2]
		Control Burbank N=33	E12 N=33	E24 N=33		
ALA	Mean	703.8	718.2	675.4	100.0-1335	392.0-952.0
	Range	323.0-1258	429.5-1053	370.6-1052		
	APV		0.7852	0.7574		
ARG	Mean	945.1	1080	969.4	367.6-1922	700.0-1383
	Range	508.0-1731	490.3-2159	530.3-1498		
	APV		0.5836	0.7770		
ASP+ ASN	Mean	5391	2846	2747	100.0-13340	3385-7380
	Range	2090-8228	1874-5114	1786-4708		
	APV		0.0126	0.0108		
GLU+ GLN	Mean	3051	4843	4615	568.4-7011	2915-6035
	Range	1840-3982	3517-7376	3462-6450		
	APV		0.0153	0.0279		
GLY	Mean	1459	1329	1328	100.0-2865	500.0-1990
	Range	307.0-3548	489.0-2885	468.0-2860		
	APV		0.7093	0.7987		
HIS	Mean	372.9	425.9	415.9	100.0-760.7	133.0-469.0
	Range	100.0-761.5	170.4-990.5	178.0-760.2		
	APV		0.5621	0.9012		
ILE	Mean	687.2	705.2	723.6	144.8-1346	525.0-953.0
	Range	372.0-911.7	405.5-1266	448.1-963.9		
	APV		0.8494	0.7007		
LEU	Mean	1228	1266	1251	476.9-2174	685.0-1383
	Range	530.0-1783	803.7-2024	594.6-1954		
	APV		0.7951	0.8154		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.
[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 9 (Continued): Russet Burbank Total Amino Acids (parts per million)[1]

Total Amino Acid (ppm)					TI	CLR[2]
		Control Burbank N=33	E12 N=33	E24 N=33		
LYS	Mean	893.9	1111	1034	100.0-3363	687.0-1368
	Range	560.0-1304	763.3-1795	741.0-1356		
	APV		0.3594	0.6609		
MET	Mean	367.5	418.5	398.0	100.0-593.3	300.0-500.0
	Range	185.6-616.7	228.3-826.4	146.6-702.1		
	APV		0.6368	0.8296		
PHE	Mean	787.1	798.6	763.5	397.1-1395	552.0-1087
	Range	417.0-1150	541.5-1247	453.4-1050		
	APV		0.8665	0.8266		
PRO	Mean	718.5	767.8	711.0	100.0-1492	355.0-1464
	Range	321.0-1415	487.0-1425	481.0-1230		
	APV		0.5091	0.8145		
SER	Mean	547.6	575.1	587.6	100.0-1362	500.0-1022
	Range	214.0-817.0	219.5-984.6	303.7-965.0		
	APV		0.7322	0.7240		
THR	Mean	646.2	658.5	680.9	226.0-1315	500.0-1022
	Range	219.8-1330.6	298.8-1346.6	330.5-1271.2		
	APV		0.8247	0.7064		
TRP	Mean	199.8	204.4	212.6	114.7-434.7	436.0-855.0
	Range	182-224	163-237	181-256		
	APV		0.7656	0.4623		
TYR	Mean	562.4	620.1	592.9	100.0-1523	457.0-942.0
	Range	327.9-792.2	266.3-989.2	254.5-977.0		
	APV		0.7383	0.8032		
VAL	Mean	1176	1076	1043	100.0-1860	752.0-1450
	Range	564.0-2012	612.0-1519	619.4-1520		
	APV		0.7857	0.9682		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 10: Russet Burbank Glycoalkaloids (mg/100 g)[1]

Glycoalkaloids (mg/100g)				TI	CLR[2]
	Control Burbank N=33	E12 N=33	E24 N=33		
Mean	7.404	5.678	6.845	0.000-33.05	3.20 – 210.4
Range	2.74-18.59	1.69-10.53	1.45-33.81		
P-value		0.1707	0.8174		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. P-values indicating significant differences with controls are underlined.
[2]Literature ranges from Kozukue *et al.* (2008).

Table 11: Russet Burbank Sugars (percent fresh weight)[1]

Fructose + Glucose (%)						
		Burbank Control	E12	E24	TI	CLR[2]
Fructose + Glucose Fresh	Mean	0.195	0.160	0.160	0.000-0.307	0.00-0.80
	Range	0.053-0.357	0.069-0.339	0.048-0.297		
	P-Value		0.1326	0.2298		
	N	12	12	12		
Fructose + Glucose Month 1	Mean	0.162	0.107	0.115	0.000-0.307	0.00-0.80
	Range	0.053-0.393	0.034-0.251	0.022-0.275		
	P-Value		0.0006	0.0019		
	N	21	21	21		
Fructose + Glucose Month 3	Mean	0.290	0.249	0.276	0.000-0.307	0.00-0.80
	Range	0.117-0.660	0.081-0.580	0.090-0.653		
	P-Value		0.4164	0.7803		
	N	27	27	27		
Fructose + Glucose Month 5	Mean	0.190	0.145	0.184	0.000-0.307	0.00-0.80
	Range	0.092-0.630	0.085-0.489	0.069-0.556		
	P-Value		0.3090	0.4307		
	N	21	21	21		
Sucrose (%)						
		Burbank Control	E12	E24	TI	CLR
Sucrose Fresh	Mean	0.179	0.213	0.178	0.000-0.315	0.05-0.32
	Range	0.113-0.288	0.134-0.299	0.116-0.259		
	P-Value		0.1284	0.9641		
	N	12	12	12		
Sucrose Month 1	Mean	0.168	0.132	0.133	0.000-0.315	0.05-0.32
	Range	0.103-0.252	0.060-0.258	0.065-0.2461		
	P-Value		0.0024	0.0028		
	N	21	21	21		
Sucrose Month 3	Mean	0.079	0.073	0.067	0.000-0.315	0.05-0.32
	Range	0.047-0.121	0.034-0.126	0.040-0.112		
	P-Value		0.5819	0.1565		
	N	27	27	27		
Sucrose Month 5	Mean	0.079	0.088	0.076	0.000-0.315	0.05-0.32
	Range	0.035-0.127	0.051-0.141	0.036-0.143		
	P-Value		0.6869	0.9047		
	N	21	21	21		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. P-values indicating significant differences with controls are underlined.

[2]Literature ranges from Blenkinsop *et al.* (2002); Matsuura-Endo *et al.* (2006); Menendez *et al.* (2002).

Table 12: Russet Burbank Acrylamide Levels in Processed Product[1]

Acrylamide (ppb)		Burbank Control	E12	E24
Fresh	Mean	493.8	162.4	158.9
2010 & 2011	Range	286-770	95-298	63-333
	P-value	—	<0.0001	<0.0001
	N	36	36	36
Month 2	Mean	778.9	237.2	280.4
2009	Range	619-950	209-251	219-389
	P-value	—	<0.0001	<0.0001
	N	9	9	9
Month 3	Mean	814.3	314.5	329.8
2010 & 2011	Range	502-1240	169-524	171-495
	P-value	—	<0.0001	<0.0001
	N	24	24	24
Month 5	Mean	230.4	82.0	93.8
2009	Range	207-260	67-97	79-109
	P-value	—	<0.0001	<0.0001
	N	9	9	9
Month 6	Mean	401.3	191.5	220.3
2010	Range	234-776	105-272	150-321
	P-value	—	<0.0001	<0.0001
	N	12	12	12
Month 7	Mean	661.0	192.0	202.7
2009	Range	598-693	165-217	187-221
	P-value	—	<0.0001	<0.0001
	N	9	9	9

[1]P-values indicating significant differences with controls are underlined.

Compositional Analysis of Event E12

Tubers of the event E12 were equivalent to those of untransformed Russet Burbank except for the following expected changes:

- E12 tubers contained, on average, 618 parts per million (ppm) free ASN as compared to controls, which contained 2421 ppm (APV = 0.1148). This change was not statistically significant, although linked to a reduced acrylamide-forming potential.

- E12 tubers contained, on average, 2846 ppm total ASN + ASP as compared to controls, which contained 5391 ppm (APV = 0.0126). The new level fits within the tolerance interval and is still normal for potato.

- E12 tubers contained, on average, 4843 ppm total GLN + GLU as compared to controls, which contained 3051 ppm (APV = 0.0153). The new levels are still normal for potato.

- E12 tubers at 1 month after harvest contained, on average, 0.107% of the reducing sugars glucose plus fructose as compared to controls, which contained 0.162% (P-value = 0.0006). The decrease in reducing sugars was expected from silencing the promoters associated with the *R1* and *PhL* genes. The new levels are still normal for potato.

- French fries from potato strips from fresh E12 potato tubers contained 162.4 ppb acrylamide as compared to control, which had 493.8 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in free ASN, total ASN + ASP, and total GLN + GLU because as non-essential amino acids they can be synthesized by the body. The observed decrease in reducing sugars falls within the normal range for potatoes. However, the combination of lower ASN and reducing sugars contribute to the reduced potential of E12 to form acrylamide and would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

The following change was *observed*:

- E12 tubers at 1 month after harvest contained, on average, 0.132% of sucrose as compared to controls, which contained 0.168% (P-value = 0.0024). The new levels are still normal for potato.

This change results in a very small modification in the composition of Russet Burbank E12 that is still standard for potato.

It can be concluded that tubers of E12 are substantially equivalent to untransformed Russet Burbank except for: (1) lowered free ASN, (2) lower levels of total ASP + ASN, (3) higher levels of total GLU + GLN, (4) the expected decreased levels of the reducing sugars glucose plus fructose, (5) reduced sucrose, and (6) reduced acrylamide as expected and intended. In all cases, these compositional changes fall within the normal range for potatoes.

Compositional Analysis of Russet Burbank and Event E24

Tubers of event E24 were equivalent to those of untransformed Russet Burbank except for the following expected changes:

- E24 tubers contained, on average, 592 parts per million (ppm) free ASN as compared to controls, which contained 2421 ppm (APV = 0.1088). This change was not statistically significant, although linked to a reduced acrylamide-forming potential.

- E24 tubers contained, on average, 2747 ppm total ASN + ASP as compared to controls, which contained 5391 ppm (APV = 0.0108). The new level fits within the tolerance interval and is still normal for potato.

- E24 tubers contained, on average, 4615 ppm total GLN + GLU as compared to controls, which contained 3051 ppm (APV = 0.0279). The new levels are still normal for potato.

- E24 tubers at 1 month after harvest contained, on average, 0.115% of the reducing sugars glucose plus fructose as compared to controls, which contained 0.162% (P-value = 0.0019). The decrease in reducing sugars was expected from silencing the promoters associated with the *R1* and *PhL* genes. The new levels are still normal for potato.

- French fries from potato strips from fresh E24 potato tubers contained 158.9 ppb acrylamide as compared to controls, which had 493.8 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in free ASN, total ASN + ASP, and total GLN + GLU because as non-essential amino acids they can be synthesized by the body. The observed decrease in reducing sugars falls within the normal range for potatoes. However, the combination of lower ASN and reducing sugars contribute to the reduced potential of E24 to for acrylamide and would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

The following change was *observed*:

- E24 tubers at 1 month after harvest contained, on average, 0.133% of sucrose as compared to controls, which contained 0.168% (P-value = 0.0028). The new levels are still normal for potato.

This change results in a very small modification in the composition of Russet Burbank E24 that is still standard for potato.

It can be concluded that tubers of E24 are substantially equivalent to untransformed Russet Burbank except for: (1) lowered free ASN, (2) lower levels of total ASP + ASN, (3) higher levels of total GLU + GLN, (4) the expected decreased levels of the reducing sugars glucose plus fructose, (5) reduced sucrose, and (6) reduced acrylamide as expected and intended. In all cases, these compositional changes fall within the normal range for potatoes.

Compositional Analysis of Atlantic Events and Controls

Data on the biochemical composition of Atlantic events and controls, which represent the mean, range, p-value and adjusted p-values, are shown in **Tables 13** (key proximates, vitamins, and minerals), **14** (free amino acids), **15** (total amino acids), **16** (glycoalkaloids), **17** (sugars), and **18** (acrylamide in fried potato chips).

Table 13: Atlantic Proximates, Vitamins, and Minerals[1]

Compound						TI	CLR[2]
		Control Atlantic N=47	J3 N=47	J55 N=47	J78 N=47		
Protein (%)	Mean	2.313	2.300	2.259	2.391	1.258-3.594	0.70-4.60
	Range	1.58-2.93	1.56-3.32	1.41-2.86	1.60-3.14		
	APV		0.9028	0.5659	0.6000		
Fat (%)	Mean	0.131	0.122	0.116	0.129	0.000-0.341	0.02-0.20
	Range	0.04-0.60	0.02-0.50	0.02-0.40	0.02-0.40		
	APV		NA	NA	NA		
Ash(%)	Mean	1.216	1.227	1.257	1.268	0.391-1.888	0.44-1.90
	Range	0.49-1.59	0.10-2.04	0.63-2.12	0.48-1.92		
	APV		0.7664	0.5720	0.7144		
Crude Fiber (%)	Mean	0.344	0.342	0.342	0.339	0.142-0.690	0.17-3.50
	Range	0.216-0.436	0.215-0.457	0.212-0.46	0.157-0.447		
	APV		0.8415	0.8608	0.7465		
Carbohydrates (%)	Mean	20.39	20.66	20.50	20.14	12.29-25.92	13.30-30.53
	Range	17.10-24.30	17.40-25.50	17.40-25.60	16.20-25.40		
	APV		1.0000	0.9540	0.6230		
Calories (kcal/ 100g)	Mean	91.71	92.72	91.90	91.11	59.09-114.9	70.00-110.2
	Range	80.20-107.7	78.30-112.1	78.50-110.8	75.90-110.4		
	APV		1.0000	0.9012	0.8409		
Moisture (%)	Mean	75.99	75.73	75.90	76.10	70.28-83.91	63.20-86.90
	Range	72.00-79.40	70.90-79.50	71.10-79.30	71.40-80.20		
	APV		1.0000	0.9237	0.7296		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992), Talburt and Smith (1987).

Table 13 (Continued): Atlantic Proximates, Vitamins, and Minerals[1]

Compound						TI	CLR[2]
		Control Atlantic N=47	J3 N=47	J55 N=47	J78 N=47		
B_3 (mg/ 100g)	Mean	1.935	1.959	2.002	2.020	0.922-3.123	0.09-3.10
	Range	1.40-2.65	1.39-2.81	1.50-2.82	1.25-3.38		
	APV		0.8217	0.5292	0.6738		
B_6 (mg/ 100g)	Mean	0.124	0.117	0.117	0.127	0.059-0.192	0.13-0.41
	Range	0.09-0.16	0.09-0.16	0.09-0.16	0.09-0.17		
	APV		0.0024	0.0012	1.0000		
Vitamin C (mg/ 100g)	Mean	20.66	21.31	21.91	22.16	0.000-129.3	1.00-54.00
	Range	9.22-38	8.11-35.3	10.94-34.2	8.65-36.1		
	APV		0.9353	0.5178	1.0000		
Cu (ppm)	Mean	1.128	1.064	1.012	1.081	0.111-2.236	0.15-7.00
	Range	0.67-1.78	0.51-1.82	0.50-1.91	0.66-1.76		
	APV		0.8378	0.3012	0.8204		
Mg (ppm)	Mean	220.0	216.7	212.8	221.8	101.5-371.5	112.50-550.00
	Range	158.0-291.0	105.0-292.0	122.0-284.0	152.0-306.0		
	APV		0.8033	0.4692	0.7984		
K (ppm)	Mean	4870	4819	4903	4962	2711-6882	3500.00-6250.00
	Range	3680-6650	2442-6951	2766-6515	3710-6680		
	APV		0.8675	0.9839	0.8779		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper. Adjusted P-values indicating significant differences with controls are underlined.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992) , Talburt and Smith (1987).

Table 14: Atlantic Free Amino Acids (parts per million)[1]

Free Amino Acid (ppm)		Control Atlantic N=47	J3 N=47	J55 N=47	J78 N=47	TI[2]	CLR[3]
ALA	Mean	103.0	103.5	102.7	103.7	100.0-208.2	12.00-236.0
	Range	100.0-214.0	100.0-264.8	100.0-202.2	100.0-244.6		
	APV		NA	NA	NA		
ARG	Mean	439.2	489.6	454.5	523.4	100.0-1068	120.0-1472
	Range	157.8-798.3	157.2-817.0	100.0-771.5	100.0-907.0		
	APV		1.0000	1.0000	0.7597		
ASN	Mean	2268	516.2	502.4	551.1	500.0-4952	312.0-6980
	Range	300.0-3710	203.0-992.0	187.0-802.0	234.0-1870		
	APV		0.0260	0.0499	0.0548		
ASP	Mean	554.0	525.3	517.8	561.3	100.0-1411	64.00-752.0
	Range	235.0-1974	239.0-1928	224.0-2002	252.0-2206		
	APV		0.7536	0.6056	1.0000		
GLN	Mean	1194	1879	1934	1932	100.0-3449	440.0-5396
	Range	425.0-3504	605.0-3686	565.0-4128	582.0-4268		
	APV		0.0772	0.0654	0.0969		
GLU	Mean	728.2	762.3	748.8	754.6	100.0-1192	450.0-752.0
	Range	344.0-1357	317.2-1238	334.4-1428	331.2-1486		
	APV		1.0000	1.0000	1.0000		
GLY	Mean	100.9	100.7	100.5	100.9	100.0-116.1	2.00-140.0
	Range	100.0-157.9	100.0-138.9	100.0-128.9	100.0-139.8		
	APV		NA	NA	NA		
HIS	Mean	102.4	100.8	102.4	100.5	100.0-136.9	34.00-656.0
	Range	100.0-153.0	100.0-130.0	100.0-152.0	100.0-120.0		
	APV		NA	NA	NA		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010). GLN high level reported as 5396 ppm from the mean of 4 sites (Davies 1977) omitting a level of 18,244 ppm from a single site, which appeared to be an outlier.

Table 14 (Continued): Atlantic Free Amino Acids (parts per million)[1]

Free Amino Acid (ppm)						TI[2]	CLR[3]
		Control Atlantic N=47	J3 N=47	J55 N=47	J78 N=47		
ILE	Mean	103.4	100.1	101.1	101.4	100.0-217.8	28.00-330.0
	Range	100.0-164.6	100.0-108.5	100.0-125.9	100.0-169.1		
	APV		NA	NA	NA		
LEU	Mean	100.0	100.0	100.0	100.0	100.0-173.9	16.00-262.0
	Range	100.0-100.0	100.0-100.0	100.0-100.0	100.0-100.0		
	APV		NA	NA	NA		
LYS	Mean	115.5	107.3	109.6	110.7	100.0-557.3	18.00-638.0
	Range	100.0-295.9	100.0-189.1	100.0-235.4	100.0-297.7		
	APV		NA	NA	NA		
MET	Mean	113.4	109.5	110.7	109.1	100.0-191.6	18.00-216.0
	Range	100.0-170.0	100.0-163.0	100.0-175.0	100.0-156.0		
	APV		NA	NA	NA		
PHE	Mean	103.4	100.4	100.5	100.4	100.0-294.9	0.00-408.0
	Range	100.0-151.7	100.0-133.2	100.0-112.0	100.0-121.5		
	APV		NA	NA	NA		
PRO	Mean	240.3	248.5	250.3	256.0	100.0-630.5	0.00-968.0
	Range	100.0-1090.0	100.0-904.0	100.0-974.0	100.0-1220		
	APV		NA	NA	NA		
SER	Mean	106.0	101.4	103.6	105.0	100.0-198.1	30.00-256.0
	Range	100.0-166.0	100.0-123.0	100.0-198.0	100.0-179.0		
	APV		NA	NA	NA		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010).

Table 14 (Continued): Atlantic Free Amino Acids (parts per million)[1]

Free Amino Acid (ppm)		Control Atlantic N=47	J3 N=47	J55 N=47	J78 N=47	TI[2]	CLR[3]
THR	Mean	106.5	106.6	107.5	108.8	100.0-219.8	28.00-286.00
	Range	100.0-241.7	100.0-233.5	100.0-323.7	100.0-339.0		
	APV		NA	NA	NA		
TRP	Mean	100.7	100.6	100.4	100.3	100.0-113.1	14.00-348.00
	Range	100.0-171.0	100.0-135.0	100.0-124.0	100.0-131.0		
	APV		NA	NA	NA		
TYR	Mean	101.9	100.0	100.9	100.7	100.0-225.2	18.00-638.00
	Range	100.0-145.0	100.0-100.0	100.0-139.0	100.0-150.2		
	APV		NA	NA	NA		
VAL	Mean	147.0	134.5	137.0	143.1	100.0-460.6	30.00-740.00
	Range	100.0-278.0	100.0-326.2	100.0-258.0	100.0-251.0		
	APV		0.0118	0.0373	1.0000		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010).

Table 15: Atlantic Total Amino Acids (parts per million)[1]

Total Amino Acid (ppm)						TI	CLR[2]
		Control Atlantic N=47	J3 N=47	J55 N=47	J78 N=47		
ALA	Mean	505.2	550.7	510.3	557.8	100.0-1335	392.0-952.0
	Range	100.0-873.0	100.0-922.0	100.0-904.0	100.0-960.0		
	APV		0.2927	1.0000	0.3308		
ARG	Mean	1109	1198	1130	1233	367.6-1922	700.0-1383
	Range	702.0-1464	659.8-1645	521.3-1634	690.5-1710		
	APV		0.3598	1.0000	0.2451		
ASP+ ASN	Mean	5986	3721	3590	3789	100.0-13340	3385-7380
	Range	296.0-12215	2110-7946	1980-7178	2250-7814		
	APV		0.1591	0.1358	0.1714		
GLU+ GLN	Mean	3729	4595	4628	4624	568.4-7011	2915-6035
	Range	1580-6474	1763-7966	1842-7820	1641-7386		
	APV		0.2140	0.1931	0.1977		
GLY	Mean	986.7	1083	956.3	1061	100.0-2865	500.0-1990
	Range	474.0-2383	426.1-3163	487.5-2290	598.8-2701		
	APV		0.3847	1.0000	0.3933		
HIS	Mean	296.3	283.4	297.5	306.4	100.0-760.7	133.0-469.0
	Range	100.0-683.5	100.0-459.0	100.0-490.0	100.0-471.0		
	APV		0.6403	1.0000	0.7345		
ILE	Mean	780.2	828.7	771.4	813.6	144.8-1346	525.0-953.0
	Range	212.6-1374	244.3-1674	249.0-1156	248.3-1580		
	APV		0.3589	1.0000	0.3853		
LEU	Mean	1353	1422	1343	1435	476.9-2174	685.0-1383
	Range	843.5-1867	771.6-2181	607.8-1880	766.1-2042		
	APV		0.4487	1.0000	0.4321		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 15 (Continued): Atlantic Total Amino Acids (parts per million)[1]

Total Amino Acid (ppm)						TI	CLR[2]
		Control Atlantic N=47	J3 N=47	J55 N=47	J78 N=47		
LYS	Mean	1601	1737	1715	1746	100.0-3363	687.0-1368
	Range	647.8-4034	716.0-4407	765.5-4581	679.1-4446		
	APV		0.2754	0.6618	0.3990		
MET	Mean	312.6	319.7	313.7	329.0	100.0-593.3	300.0-500.0
	Range	169.3-511.0	100.0-499.0	100.0-520.0	100.0-530.0		
	APV		0.7163	0.9970	0.4430		
PHE	Mean	927.1	974.2	946.5	969.7	397.1-1395	552.0-1087
	Range	555.1-1240	556.3-1290	510.1-1260	523.2-1340		
	APV		0.4102	1.0000	0.4678		
PRO	Mean	870.8	943.9	914.7	957.9	100.0-1492	355.0-1464
	Range	409.3-2090	340.1-1850	326.3-1960	496.6-2080		
	APV		0.2876	0.9755	0.2784		
SER	Mean	670.1	717.7	704.0	730.8	100.0-1362	500.0-1022
	Range	256.1-1130	271.5-1190	155.1-1150	252.3-1190		
	APV		0.3548	0.8755	0.2836		
THR	Mean	807.6	903.4	858.0	877.1	226.0-1315	436.0-855.0
	Range	356.7-1234	399.4-1398	357.3-1219	375.4-1180		
	APV		0.3212	1.0000	0.3421		
TYR	Mean	843.4	872.5	850.7	894.0	100.0-1523	457.0-942.0
	Range	375.5-1451	335.4-1613	270.8-1520	318.1-1520		
	APV		0.6265	1.0000	0.4160		
VAL	Mean	810.3	855.3	805.5	875.8	100.0-1860	752.0-1450
	Range	275.7-1380	175.3-1400	221.1-1390	269.4-1490		
	APV		0.4321	1.0000	0.3117		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 16: Atlantic Glycoalkaloids (mg/100 g)[1]

Glycoalkaloids (mg/100g)					TI	CLR[2]
	Control Atlantic N=47	J3 N=47	J55 N=47	J78 N=47		
Mean	6.274	5.723	5.864	6.084	0.000-33.05	3.20 – 210.40
Range	2.09-11.40	1.62-11.67	1.83-11.12	1.65-14.27		
P-value		0.2393	0.4833	0.7198		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. P-values indicating significant differences with controls are underlined.
[2]Literature ranges from Kozukue *et al.* (2008).

Table 17: Atlantic Sugars (percent fresh weight)[1]

Fructose + Glucose (%)							
		Atlantic Control	J3	J55	J78	TI	CLR[2]
Fructose + Glucose Fresh	Mean	0.062	0.049	0.048	0.048	0.000-0.307	0.00-0.80
	Range	0.003-0.23	0.002-0.385	0.003-0.263	0.002-0.237		
	P-Value		0.0047	0.0161	0.0454		
	N	47	47	47	47		
Fructose + Glucose Month 1	Mean	0.123	0.095	0.092	0.107	0.000-0.307	0.00-0.80
	Range	0.031-0.278	0.033-0.216	0.031-0.267	0.033-0.268		
	P-Value		0.0028	0.0004	0.1012		
	N	47	46	46	46		
Fructose + Glucose Month 2	Mean	0.070	0.061	0.066	0.077	0.000-0.307	0.00-0.80
	Range	0.028-0.182	0.025-0.172	0.028-0.178	0.029-0.229		
	P-Value		0.2446	0.6004	0.9998		
	N	26	26	26	26		
Sucrose (%)							
		Atlantic Control	J3	J55	J78	TI	CLR
Sucrose Fresh	Mean	0.162	0.165	0.166	0.166	0.000-0.315	0.05-0.32
	Range	0.057-0.341	0.052-0.25	0.075-0.366	0.045-0.309		
	P-Value		0.9772	0.9444	0.9246		
	N	47	47	47	47		
Sucrose Month 1	Mean	0.126	0.132	0.123	0.128	0.000-0.315	0.05-0.32
	Range	0.015-0.224	0.016-0.247	0.017-0.21	0.020-0.227		
	P-Value		0.9295	0.9995	0.9834		
	N	47	46	46	46		
Sucrose Month 2	Mean	0.132	0.142	0.140	0.131	0.000-0.315	0.05-0.32
	Range	0.082-0.202	0.073-0.295	0.084-0.286	0.022-0.245		
	P-Value		0.6559	0.8069	0.9994		
	N	26	26	26	26		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. P-values indicating significant differences with controls are underlined.

[2]Literature ranges from Blenkinsop *et al.* (2002); Matsuura-Endo *et al.* (2006); Menendez *et al.* (2002).

Table 18: Atlantic Acrylamide Levels in Processed Product (parts per billion)[1]

Acrylamide (ppb)		Atlantic Control	J3	J55	J78
Fresh 2010 & 2011	Mean	842.7	278.7	233.4	251.1
	Range	113-4540	37-4120	39-1400	36-1280
	P-value	—	<0.0001	<0.0001	<0.0001
	N	47	47	47	47
Month 2 2010 & 2011	Mean	1681.4	529.8	534.3	558.4
	Range	181-9840	80-2110	80-2850	82-1930
	P-value	—	<0.0001	<0.0001	<0.0001
	N	47	47	47	47

[1]P-values indicating significant differences with controls are underlined.

Compositional Analysis of Atlantic and Event J3

All discussion for the Atlantic variety and event J3 relates to observations summarized from field trials conducted in 2010 and 2011. Compositional equivalence was tested only in the fresh tubers, although reducing sugars and acrylamide were tested during storage to show trait efficacy.

Tubers of event J3 were equivalent to those of untransformed Atlantic except for the following expected changes:

- J3 tubers contained, on average, 516 parts per million (ppm) free ASN as compared to controls, which contained 2268 ppm (APV = 0.0260). This change was expected and is linked to a reduced acrylamide-forming potential.

- J3 tubers contained, on average, 0.049% of the reducing sugars glucose plus fructose as compared to controls, which contained 0.062% (P-value = 0.0047). The decrease in reducing sugars was expected from silencing the promoters associated with the *R1* and *PhL* genes. The new levels are still normal for potato.

- After one month of storage, J3 tubers contained, on average, 0.095% of the reducing sugars glucose plus fructose as compared to controls, which contained 0.123% (P-value = 0.0028). The decrease in reducing sugars was expected from silencing the promoters associated with the *R1* and *PhL* genes. The new levels are still normal for potato.

- Potato chips from fresh J3 tubers contained 278.7 ppb acrylamide as compared to control, which had 842.7 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in ASN because as a non-essential amino acid it can be synthesized by the body. The observed decrease in reducing sugars falls within the normal range for potatoes. However, the combination of lower ASN and reducing sugars contribute to the reduced potential of J3 to form acrylamide and would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

The following changes were *observed*:

- J3 tubers contained, on average, 0.117 mg/100 g pyridoxine (vitamin B6) as compared to controls, which contained 0.124 mg/100 g (APV = 0.0024). The new levels are still normal for potato.

- J3 tubers contained, on average, 134 ppm free VAL as compared to controls, which contained 147 ppm (APV = 0.0118). The new levels are still normal for potato.

These two changes result in very small modifications to the vitamin and amino acid composition of Atlantic J3 that are still standard for potato and will not significantly alter potential dietary intake levels.

It can be concluded that tubers of J3 are substantially equivalent to untransformed Atlantic tubers except for: (1) lowered free ASN, (2) the expected decreased levels of the reducing sugars glucose plus fructose, (3) reduced acrylamide as expected and intended, (4) small decreases in pyridoxine as compared to controls, and (5) a slight decrease of free VAL as compared to controls. In all cases, these compositional changes fall within the normal range for potatoes.

Compositional Analysis of Atlantic and Event J55

All discussion for the Atlantic variety and event J55 relates to observations summarized from field trials conducted in 2010 and 2011. Compositional equivalence was tested only in the fresh tubers, although reducing sugars and acrylamide were tested during storage to show trait efficacy.

Tubers of the event J55, were equivalent to those of untransformed Atlantic except for the following expected changes:

- J55 tubers contained, on average, 502 parts per million (ppm) free ASN as compared to controls, which contained 2268 ppm (APV = 0.0499). This change was expected and is linked to a reduced acrylamide-forming potential.

- J55 tubers contained, on average, 0.048% of the reducing sugars glucose plus fructose as compared to controls, which contained 0.062% (P-value = 0.0161). The decrease in reducing sugars was expected from silencing the promoters associated with the *R1* and *PhL* genes. The new levels are still normal for potato.

- After one month of storage, J55 tubers contained, on average, 0.092% of the reducing sugars glucose plus fructose as compared to controls, which contained 0.123% (P-value = 0.0004). The decrease in reducing sugars was expected from silencing the promoters associated with the *R1* and *PhL* genes. The new levels are still normal for potato.

- Potato chips from fresh J55 tubers contained 233.4 ppb acrylamide as compared to control, which had 842.7 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in free ASN because as a non-essential amino acid it can be synthesized by the body. The observed decrease in reducing sugars falls within the normal range for potatoes. However, the combination of lower ASN and reducing sugars contribute to the reduced potential of J55 to form acrylamide and would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

The following changes were *observed*:

- J55 tubers contained, on average 0.117 mg/100 g pyroxidine (vitamin B_6) as compared to controls, which contained 0.124 mg/100 g (APV = 0.0012). The new levels are still normal for potato.

- J55 tubers contained, on average, 137 ppm free VAL as compared to controls, which contained 147 ppm (APV = 0.0373). The new levels are still normal for potato.

These two changes result in very small modifications to the vitamin and amino acid composition of Atlantic J55 that are still standard for potato and will not significantly alter potential dietary intake levels

It can be concluded that tubers of Atlantic J55 are substantially equivalent to untransformed Atlantic tubers except for: (1) lowered free ASN, (2) the expected decreased levels of the reducing sugars glucose plus fructose, (3) reduced acrylamide as expected and intended, (4) small decreases in pyroxidine as compared to controls, and (5) a slight decrease in free VAL as compare to controls. In all cases, these compositional changes fall within the normal range for potatoes.

Compositional Analysis of Atlantic and Event J78

All discussion for the Atlantic variety and event J78 relates to observations summarized from field trials conducted in 2010 and 2011. Compositional equivalence was tested only in the fresh tubers, although reducing sugars and acrylamide were tested during storage to show trait efficacy.

Tubers of the event J78, were equivalent to those of untransformed Atlantic except for the following expected changes:

- J78 tubers contained, on average, 551 parts per million (ppm) free ASN as compared to controls, which contained 2268 ppm (APV = 0.0548). This change was expected and is linked to a reduced acrylamide-forming potential. Although the APV is slightly above our cutoff for significance of APV < 0.0500, it is worth noting the large difference between J78 and the controls.

- J78 tubers contained, on average, 0.048% of the reducing sugars glucose plus fructose as compared to controls, which contained 0.062% (P-value = 0.0454). The decrease in reducing sugars was not expected because J78 does not contain the silencing cassette for the promoters associated with the *R1* and *PhL* genes. The new levels are still normal for potato.

- Potato chips from fresh J78 tubers contained 251.1 ppb acrylamide as compared to control, which had 842.7 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in free ASN because as a non-essential amino acid it can be synthesized by the body. The observed decrease in reducing sugars falls within the normal range for potatoes. However, the combination of lower ASN and reducing sugars contribute to the reduced potential of J78 to form acrylamide and would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

There were no other changes observed for event J78.

It can be concluded that tubers of J78 are substantially equivalent to untransformed Atlantic tubers except for: (1) lowered free ASN, (2) the decreased levels of the reducing sugars glucose plus fructose was not expected, and (3) reduced acrylamide as expected and intended. In all cases, these compositional changes fall within the normal range for potatoes.

Compositional Analysis of G Event and Control

Data on the biochemical composition of G control and event G11 are shown in **Tables 19** (key proximates, vitamins, and minerals), **20** (free amino acids), **21** (total amino acids), **22** (glycoalkaloids), and **23** (reducing sugars), and **24** (acrylamide in fried product). The analysis was based on 2 tubers per rep, 4 reps per location (RCB - Randomized Complete Block field experiment), and 6 locations, all from 2010 field trials. In addition, some acrylamide data were collected in 2009 and 2011.

Table 19: G Variety Proximates, Vitamins, and Minerals[1]

Compound					
		G Control N=20	G11 N=20	TI	CLR[2]
Protein (%)	Mean	2.54	2.44	1.258-3.594	0.70-4.60
	Range	1.70-3.07	1.58-3.08		
	APV		0.361		
Fat(%)	Mean	0.21	0.18	0.000-0.341	0.02-0.20
	Range	0.10-0.60	0.10-0.30		
	APV		0.903		
Ash (%)	Mean	1.16	1.28	0.391-1.888	0.44-1.90
	Range	0.81-1.44	1.03-1.54		
	APV		0.278		
Crude Fiber (%)	Mean	0.39	0.38	0.142-0.690	0.17-3.50
	Range	0.30-0.47	0.10-0.53		
	APV		0.935		
Carbohydrates (%)	Mean	20.62	20.48	12.29-25.92	13.30-30.53
	Range	16.20-26.30	16.00-25.00		
	APV		0.829		
Calories (kcal/ 100 g)	Mean	94.32	93.1	59.09-114.9	70.00-110.2
	Range	78.40-115.0	78.00-108.0		
	APV		0.841		
Moisture (%)	Mean	75.48	75.64	70.28-83.91	63.20-86.90
	Range	70.10-79.80	71.40-79.50		
	APV		0.871		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992) , Talburt and Smith (1987).

Table 19 (Continued): G Variety Proximates, Vitamins, and Minerals[1]

Compound					
		G Control N=20	G11 N=20	TI	CLR[2]
B3 (mg/ 100 g)	Mean	1.86	1.93	0.922-3.123	0.09-3.10
	Range	1.61-2.18	1.68-2.18		
	APV		0.784		
B6 (mg/ 100 g)	Mean	0.18	0.19	0.059-0.192	0.13-0.41
	Range	0.17-0.20	0.15-0.23		
	APV		0.359		
Vitamin C (mg/ 100 g)	Mean	26.03	29.14	0.000-129.3	1.00-54.00
	Range	18.50-36.10	20.50-38.40		
	APV		0.418		
Cu (ppm)	Mean	1.14	1.17	0.111-2.236	0.15-7.00
	Range	0.74-1.54	0.71-1.93		
	APV		0.603		
Mg (ppm)	Mean	263.3	268.5	101.5-371.5	112.5-550.0
	Range	198.0-319.0	221.0-338.0		
	APV		0.567		
K (ppm)	Mean	4598	4792	2711-6882	3500-6250
	Range	3710-5540	3820-5690		
	APV		0.341		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper, ppm = parts per million. Adjusted P-values indicating significant differences with controls are underlined.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992) , Talburt and Smith (1987).

Table 20: G Variety Free Amino Acids (parts per million)[1]

Free amino acids (ppm)					
		G Control N=20	G11 N=20	TI[2]	CLR[3]
ALA	Mean	100	100	100.0-208.2	12.00-236.0
	Range	100-100	100-100		
	APV		NA		
ARG	Mean	609.8	626.2	100.0-1068	120.0-1472
	Range	350-973	344-1000		
	APV		0.597		
ASN	Mean	3406	794	500.0-4952	312.0-6980
	Range	2190-4100	440-942		
	APV		0.003		
ASP	Mean	304.2	330.1	100.0-1411	64.00-752.0
	Range	264-369	270-542		
	APV		0.408		
GLU	Mean	434.9	475.3	100.0-1192	450.0-742.0
	Range	280-563	405-615		
	APV		0.516		
GLN	Mean	889.4	1666	100.0-3449	440.0-5396
	Range	557-1470	954-2490		
	APV		0.017		
HIS	Mean	107.4	100	100.0-136.9	34.00-656.0
	Range	100-147	100-100		
	APV		NA		
ILE	Mean	106.7	102.1	100.0-217.8	28.00-330.0
	Range	100-147	100-134		
	APV		NA		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010). GLN high level reported as 5396 ppm from the mean of 4 sites (Davies 1977) omitting a level of 18,244 ppm from a single site, which appeared to be an outlier.

Table 20 (Continued): G Variety Free Amino Acids (parts per million)[1]

Free amino acids (ppm)					
		G Control N=20	G11 N=20	TI[2]	CLR[3]
LYS	Mean	128.6	111.6	100.0-557.3	18.00-638.0
	Range	100-210	100-186		
	APV		NA		
MET	Mean	107.6	101.7	100.0-191.6	18.00-216.0
	Range	100-136	100-115		
	APV		NA		
PHE	Mean	107.6	100	100.0-294.9	0.00-408.0
	Range	100-139	100-100		
	APV		NA		
PRO	Mean	132.9	123.3	100.0-630.5	0.00-968.0
	Range	100-307	100-232		
	APV		NA		
SER	Mean	100	100	100.0-198.1	30.00-256.0
	Range	100-100	100-100		
	APV		NA		
THR	Mean	100	100	100.0-219.8	28.00-286.0
	Range	100-100	100-100		
	APV		NA		
TRP	Mean	100	100	100.0-113.1	14.00-348.0
	Range	100-100	100-100		
	APV		NA		
TYR	Mean	101.6	100	100.0-225.2	18.00-638.0
	Range	100-124	100-100		
	APV		NA		
VAL	Mean	132.6	112.8	100.0-460.6	30.00-748.0
	Range	100-212	100-179		
	APV		NA		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010).

Table 21: G Variety Total Amino Acids (parts per million)[1]

Total amino acids (ppm)					
		G Control N=20	G11 N=20	TI	CLR[2]
ALA	Mean	794.3	868.4	100.0-1335	392-952
	Range	671-938	658-1060		
	APV		0.06		
ARG	Mean	1317	1403	367.6-1922	700-1383
	Range	982-1750	942-1800		
	APV		0.219		
ASP+ASN	Mean	5655	3173	100.0-13340	3385-7380
	Range	3940-7710	2070-6880		
	APV		0.008		
GLU+GLN	Mean	3264	4456	568.4-7011	2915-6035
	Range	2530-4080	3080-5640		
	APV		0.034		
GLY	Mean	747.1	818.4	100.0-2865	500-1990
	Range	531-951	572-1080		
	APV		0.053		
HIS	Mean	366.7	385.7	100.0-760.7	133-469
	Range	216-509	303-484		
	APV		0.445		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 21 (Continued): G Variety Total Amino Acids (parts per million)[1]

Total amino acids (ppm)					
		G Control N=20	G11 N=20	TI	CLR[2]
ILE	Mean	874.5	929.3	144.8-1346	525-953
	Range	706-1090	667-1120		
	APV		0.071		
LEU	Mean	1568	1715	476.9-2174	685-1383
	Range	1260-1880	1270-2090		
	APV		0.059		
LYS	Mean	1055	1185	100.0-3363	687-1368
	Range	700-1510	789-1580		
	APV		0.006		
MET	Mean	380.2	391.1	100.0-593.3	300 - 500
	Range	271-488	247-485		
	APV		0.463		
PHE	Mean	1029	1099	397.1-1395	552-1087
	Range	794-1310	810-1340		
	APV		0.146		
PRO	Mean	839.3	924	100.0-1492	355-1464
	Range	633-1200	665-1160		
	APV		0.035		
SER	Mean	892.2	963.3	100.0-1362	500-1022
	Range	722-1120	733-1210		
	APV		0.058		
THR	Mean	911.7	1009	226.0-1315	436-855
	Range	738-1100	736-1240		
	APV		0.055		
TRP	Mean	258.5	246.2	114.7-434.7	114-282
	Range	213-366	206-299		
	APV		0.425		
TYR	Mean	825.6	937.3	100.0-1523	457-942
	Range	604-989	684-1160		
	APV		0.053		
VAL	Mean	1082	1154	100.0-1860	752-1450
	Range	876-1310	876-1350		
	APV		0.056		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 22: G Variety Glycoalkaloids (mg/100 g)[1]

Glycoalkaloids (mg/100 g)					
		G Control N=20	G11 N=20	TI	CLR[2]
	Mean	19.82	20.32	0.000-33.05	3.20-210.40
	Range	6.17-44.4	5.81-55.3		
	P-value		0.9153		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. P-values indicating significant differences with controls are underlined.
[2]Literature ranges from Kozukue *et al.* (2008).

Table 23: G Variety Sugars (percentage fresh weight)[1]

Fructose + Glucose (%)					
		G Control	G11	TI	CLR[2]
Fructose + Glucose Fresh	Mean	0.08	0.08	0.000-0.307	0.00-0.80
	Range	0.019-0.165	0.017-0.150		
	P-Value		0.9800		
	N	40	39		
Sucrose (%)					
		G Control	G11	TI	CLR
Sucrose Fresh	Mean	0.090	0.100	0.000-0.315	0.05-0.32
	Range	0.046-0.119	0.045-0.184		
	P-Value		0.387		
	N	20	20		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. Data represent the mean levels of reducing sugars and sucrose (% FW) in samples of 3-5 tubers, using 3 measurements per event and control per trial site. P-values indicating significant differences with controls are underlined.
[2]Literature ranges from Blenkinsop *et al.* (2002); Matsuura-Endo *et al.* (2006); Menendez *et al.* (2002).

Table 24: G Variety Acrylamide Levels in Processed Product (parts per billion)[1]

Acrylamide (ppb)		G Control	G11
Fresh 2010 & 2011	Mean	1254.2	421.5
	Range	105-4320	51-1200
	P-value	—	<u><0.0001</u>
	N	25	21
Month 1 2010 & 2011	Mean	1769.0	2050.0
	Range	215-6938	463-6210
	P-value	—	<u>0.0136</u>
	N	8	4
Month 3 2010 & 2011	Mean	2370.7	971.2
	Range	57-4890	28-2200
	P-value	—	<u><0.0001</u>
	N	14	13
Month 5 2009 & 2010	Mean	1060.7	567.6
	Range	200-2550	55-1700
	P-value	—	<u>0.0470</u>
	N	7	7
Month 7 2010	Mean	729.3	302.8
	Range	296-1490	102-488
	P-value	—	0.0629
	N	4	4

[1]P-values indicating significant differences with controls are underlined.

Compositional Analysis of the G Variety and Event G11

The composition data for G11 represent the summary from 6 replicated field trials conducted in 2010. In addition some acylamide data were collected and summarized in 2009 and 2011. Tubers of event G11 were equivalent to those of the untransformed G variety except for the following expected changes:

- G11 tubers contained, on average, 794 parts per million (ppm) free ASN compared to the control, which contained 3406 ppm (APV = 0.003). This change was expected and is linked to a reduced acrylamide-forming potential.

- G11 tubers contained, on average, 1666 ppm free GLN compared to the control, which contained 889 ppm (APV = 0.017). The increase in this amide amino acid counter-balances, in part, losses in the ASN amide amino acid. The new levels are normal for potato.

- G11 tubers contained, on average, 3173 ppm total ASN + ASP compared to the control, which contained 5655 ppm (APV = 0.008). The new level fits within the tolerance interval and is still normal for potato.

- G11 tubers contained, on average, 4456 ppm GLN + GLU compared to the control, which contained 3264 ppm (APV = 0.034). The new levels are normal for potato.

- Potato chips from G11 tubers contained 421.5 ppb acrylamide compared to the control, which had 1254.2 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in free ASN and GLN, along with total ASN + ASP, and total GLN + GLU because as non-essential amino acids they can be synthesized by the body. However, the reduced potential of G11 to form acrylamide would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

The following changes were *observed*:

- G11 tubers contained, on average, 1185 ppm total LYS as compared to controls, which contained 1055 ppm (APV = 0.006). The new levels are still normal for potato.

- G11 tubers contained, on average, 924 ppm total PRO as compared to controls, which contained 839 ppm (APV = 0.035). The new levels are still normal for potato.

It can be concluded that tubers of event G11 are substantially equivalent to untransformed G tubers except for: (1) changes in ASN and GLN, (2) lower levels of total ASP + ASN, (3) higher levels of total GLN + GLU, (4) slightly increased total LYS and PRO, and (5) reduced acrylamide as expected and intended. In all cases, these compositional changes fall within the normal range for potatoes.

Compositional Analysis of H Events and Controls

Data on the biochemical composition of the H variety and events H37 and H50 are shown in **Tables 25** (key proximates, vitamins, and minerals), **26** (free amino acids), **27** (total amino acids), **28** (glycoalkaloids), **29** (reducing sugars), and **30** (acrylamide in fried product). The analysis was based on 2 tubers per rep, 4 reps per location (RCB field experiment), and 6 locations, all from 2010 field trials. In addition, some acrylamide data were collected in 2009 and 2011.

Table 25: H Variety Proximates, Vitamins, and Minerals[1]

Compound						
		H Control N=20	H37 N=20	H50 N=20	TI	CLR[2]
Protein (%)	Mean	2.49	2.37	2.36	1.258-3.594	0.70-4.60
	Range	1.23-3.15	1.67-2.94	1.34-2.85		
	APV		0.833	0.752		
Fat (%)	Mean	0.11	0.10	0.11	0.000-0.341	0.02-0.20
	Range	0.10-0.20	0.10-0.10	0.10-0.30		
	APV		NA	NA		
Ash (%)	Mean	1.15	1.16	1.13	0.391-1.888	0.44-1.90
	Range	0.47-1.71	0.65-1.62	0.87-1.42		
	APV		1.000	0.847		
Crude Fiber (%)	Mean	0.42	0.43	0.41	0.142-0.690	0.17-3.50
	Range	0.34-0.52	0.34-0.62	0.33-0.51		
	APV		0.775	0.818		
Carbohydrates (%)	Mean	18.18	18.81	19.08	12.29-25.92	13.30-30.53
	Range	13.90-21.00	16.10-21.40	15.50-22.30		
	APV		0.904	0.681		
Calories (kcal/100 g)	Mean	83.00	85.09	86.22	59.09-114.9	70.00-110.2
	Range	66.00-95.40	74.10-93.30	74.30-98.10		
	APV		0.810	0.671		
Moisture (%)	Mean	78.14	77.62	77.38	70.28-83.91	63.20-86.90
	Range	75.40-82.50	75.50-80.80	74.50-80.40		
	APV		0.916	0.607		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper, ppm = parts per million. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992), Talburt and Smith (1987).

Table 25 (Continued): H Variety Proximates, Vitamins, and Minerals[1]

Compound						
		H Control N=20	H37 N=20	H50 N=20	TI	CLR[2]
B3 (mg/100 g)	Mean	1.68	1.73	1.66	0.922-3.123	0.09-3.10
	Range	1.40-1.98	1.50-2.15	1.42-2.16		
	APV		0.901	0.844		
B6 (mg/100 g)	Mean	0.16	0.16	0.15	0.059-0.192	0.13-0.41
	Range	0.12-0.20	0.13-0.19	0.13-0.17		
	APV		1.000	1.000		
Vitamin C (mg/100 g)	Mean	28.76	29.62	29.99	0.000-129.3	1.00-54.00
	Range	19.80-37.70	21.10-39.30	23.10-38.70		
	APV		0.814	0.710		
Cu (ppm)	Mean	1.15	0.96	0.97	0.111-2.236	0.15-7.00
	Range	0.57-2.96	0.61-1.46	0.66-1.47		
	APV		0.880	0.546		
Mg (ppm)	Mean	246.1	237.4	247.8	101.5-371.5	112.5-550.0
	Range	194.0-369.0	156.0-329.0	202.0-324.0		
	APV		0.994	0.860		
K (ppm)	Mean	4562	4779	4679	2711-6882	3500-6250
	Range	4240-5120	3810-5460	4200-5040		
	APV		0.855	0.623		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value, B6 = pyridoxine hydrochlorine (vitamin B6), B3 = niacin (vitamin B3), ascorbic acid = vitamin C, Mg = magnesium, K = potassium, Cu = copper. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from , Lisinska and Leszczynski (1989), Rogan *et al.* (2000), Horton and Anderson (1992), Talburt and Smith (1987).

Table 26: H Variety Free Amino Acids (parts per million)[1]

Free Amino Acids (ppm)						
		H Control N=20	H37 N=20	H50 N=20	TI[2]	CLR[3]
ALA	Mean	101.8	100	100	100.0-208.2	12.00-236.0
	Range	100-128	100-100	100-100		
	APV		NA	NA		
ARG	Mean	869.2	977.7	930.4	100.0-1068	120.0-1472
	Range	496-1330	654-1380	523-1230		
	APV		0.412	0.68		
ASN	Mean	2805	877.5	672.7	500.0-4952	312.0-6980
	Range	657-4100	429-2750	280-1070		
	APV		<u><.0001</u>	<u><.0001</u>		
ASP	Mean	391.9	405.5	387.9	100.0-1411	64.00-752.0
	Range	286-490	325-494	315-533		
	APV		0.854	0.847		
GLU	Mean	456.6	459.8	487.3	100.0-1192	450.0-742.0
	Range	324-596	315-589	372-590		
	APV		0.966	0.424		
GLN	Mean	1680	2724	2913	100.0-3449	440.0-5396
	Range	1100-3380	1720-3620	1140-3770		
	APV		<u>0.014</u>	<u>0.006</u>		
HIS	Mean	121.6	121.3	117.2	100.0-136.9	34.00-656.0
	Range	100-163	100-211	100-171		
	APV		0.967	0.618		
ILE	Mean	140.9	128.3	117.6	100.0-217.8	28.00-330.0
	Range	100-185	100-254	100-172		
	APV		0.441	0.082		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010). GLN high level reported as 5396 ppm from the mean of 4 sites (Davies 1977) omitting a level of 18,244 ppm from a single site, which appeared to be an outlier.

Table 26 (Continued): H Variety Free Amino Acids (parts per million)[1]

Free Amino Acids (ppm)						
		H Control N=20	H37 N=20	H50 N=20	TI[2]	CLR[3]
LYS	Mean	263.1	206.8	219.9	100.0-557.3	18.00-638.0
	Range	131-385	100-325	100-342		
	APV		0.035	0.079		
MET	Mean	135.6	132.4	129.1	100.0-191.6	18.00-216.0
	Range	100-185	100-179	100-170		
	APV		0.934	0.699		
PHE	Mean	236.2	206.3	191.8	100.0-294.9	0.00-408.0
	Range	126-362	100-342	100-281		
	APV		0.473	0.227		
PRO	Mean	130.4	118.1	157.9	100.0-630.5	0.00-968.0
	Range	100-294	100-275	100-545		
	APV		NA	NA		
SER	Mean	108.9	107.2	104.3	100.0-198.1	30.00-256.0
	Range	100-141	100-147	100-133		
	APV		NA	NA		
THR	Mean	115.3	118.7	119.4	100.0-219.8	28.00-286.0
	Range	100-162	100-152	100-150		
	APV		0.857	0.641		
TRP	Mean	100.6	104.8	110.6	100.0-113.1	14.00-348.0
	Range	100-110	100-176	100-190		
	APV		NA	NA		
TYR	Mean	129.5	154.4	156.5	100.0-225.2	18.00-638.0
	Range	100-209	100-256	100-251		
	APV		0.458	0.319		
VAL	Mean	273.1	259.7	239.4	100.0-460.6	30.00-740.0
	Range	181-400	171-467	105-375		
	APV		0.933	0.441		

[1]Free amino acids included to show efficacy of reducing free ASN and thus reducing acrylamide. Many values fell below detection limits and were adjusted to the limit (100 ppm) for statistical analysis. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance. APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined.

[2]TI = Tolerance Interval.

[3] CLR = Combined Literature Range. Literature ranges are from Davies (1977), Lisinska and Leszczynski (1989), and Shepherd *et al.* (2010).

Table 27: H Variety Total Amino Acids (parts per million)[1]

Total Amino Acids (ppm)						
		H Control N=20	H37 N=20	H50 N=20	TI	CLR[2]
ALA	Mean	642.3	677.1	676.9	100.0-1335	392.0-952.0
	Range	492-771	510-826	507-891		
	APV		0.462	0.416		
ARG	Mean	1443	1580	1547	367.6-1922	700.0-1383
	Range	930-1870	1120-1990	970-1880		
	APV		0.437	0.423		
ASP+ASN	Mean	4748	2860	2655	100.0-13340	3385-7380
	Range	2680-6190	1950-4910	1700-3320		
	APV		<.0001	<.0001		
GLU+GLN	Mean	3926	5159	5443	568.4-7011	2915-6035
	Range	3090-6130	3670-6060	2940-6620		
	APV		0.05	0.018		
GLY	Mean	636.6	667.7	668.7	100.0-2865	500 .0- 1990
	Range	497-774	514-829	501-830		
	APV		0.606	0.374		
HIS	Mean	360.7	372.4	365.3	100.0-760.7	133.0-469.0
	Range	258-423	279-466	247-475		
	APV		0.607	0.839		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 27 (Continued): H Variety Total Amino Acids (parts per million)[1]

Total Amino Acids (ppm)						
		H Control N=20	H37 N=20	H50 N=20	TI	CLR[2]
ILE	Mean	794.1	811.9	808.1	144.8-1346	525.0-953.0
	Range	573-910	607-998	556-1010		
	APV		0.6	0.745		
LEU	Mean	1226	1294	1297	476.9-2174	685.0-1383
	Range	910-1520	973-1670	930-1660		
	APV		0.554	0.364		
LYS	Mean	1161	1188	1209	100.0-3363	687.0-1368
	Range	838-1360	878-1440	817-1450		
	APV		0.589	0.334		
MET	Mean	372.9	388.2	381.4	100.0-593.3	300.0 - 500.0
	Range	265-435	268-478	237-494		
	APV		0.627	0.755		
PHE	Mean	964.6	981.3	973.9	397.1-1395	552.0-1087
	Range	675-1100	681-1240	609-1270		
	APV		0.736	0.827		
PRO	Mean	682.5	717.4	767.9	100.0-1492	355.0-1464
	Range	488-993	502-1010	485-1270		
	APV		0.604	0.487		
SER	Mean	728.1	754	751.4	100.0-1362	500.0-1022
	Range	573-903	591-934	568-925		
	APV		0.612	0.582		
THR	Mean	766.3	814.9	820.9	226.0-1315	436.0-855.0
	Range	573-924	602-1060	566-1090		
	APV		0.468	0.428		
TRP	Mean	223.4	227.3	235.3	114.7-434.7	114.0-282.0
	Range	170-291	166-273	171-352		
	APV		0.71	0.453		
TYR	Mean	714.1	795.1	830.3	100.0-1523	457.0-942.0
	Range	551-824	628-970	553-1090		
	APV		0.543	0.166		
VAL	Mean	1053	1093	1080	100.0-1860	752.0-1450
	Range	776-1200	813-1300	716-1310		
	APV		0.609	0.663		

[1]TI = Tolerance Interval, CLR = Combined Literature Range, APV = adjusted P-value. Adjusted P-values indicating significant differences with controls are underlined. NA = Adjusted p-value not determined because observations near the detection limits are too low to allow an estimate of variance.

[2]Literature ranges are from Lisinska and Leszczynski (1989), Rogan *et al.* (2000), and Talley *et al.* (1984).

Table 28: H Variety Glycoalkaloids (mg/100 g)[1]

Glycoalkaloids (mg/100 g)					TI	CLR[2]
		H Control N=20	H37 N=20	H50 N=20		
	Mean	10.65	10.76	11.30	0.000-33.05	3.20-210.40
	Range	5.96-21.07	5.41-17.25	6.23-22.81		
	P-value		0.8951	0.4379		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. P-values indicating significant differences with controls are underlined.
[2]Literature ranges from Kozukue (2008).

Table 29: H Variety Sugars (percentage fresh weight)[1]

Fructose + Glucose (%)						
		H Control	H37	H50	TI	CLR[2]
Fructose + Glucose Fresh	Mean	0.06	0.05	0.04	0.000-0.307	0.00-0.80
	Range	0.016-0.104	0.015-0.071	0.013-0.078		
	P-value		0.0830	0.0550		
	N	40	40	40		
Sucrose (%)						
		H Control	H37	H50	TI	CLR
Sucrose Fresh	Mean	0.11	0.13	0.12	0.000-0.315	0.05-0.32
	Range	0.067-0.147	0.071-0.183	0.101-0.162		
	P-value		<u>0.0450</u>	0.2250		
	N	20	<u>20</u>	20		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. Data represent the mean levels of reducing sugars and sucrose (% FW) in samples of 3-5 tubers, using 3 measurements per event and control per trial site. P-values indicating significant differences with controls are underlined. [2]Literature ranges from Blenkinsop *et al.* (2002); Matsuura-Endo *et al.* (2006); Menendez *et al.* (2002).

Table 30: H Variety Acrylamide Levels in Processed Product (parts per billion)[1]

Acrylamide (ppb)		H Control	H37	H50
Fresh 2010 & 2011	Mean	531.2	223.0	174.7
	Range	65-1110	81-512	59-545
	P-value	—	<0.0001	<0.0001
	N	25	25	25
Month 1 2010 & 2011	Mean	577.0	253.0	195.6
	Range	104-1600	65-727	43-499
	P-value	—	0.0006	<0.0001
	N	8	8	8
Month 3 2010 & 2011	Mean	791.5	440.2	293.2
	Range	43-2980	43-1160	32-602
	P-value	—	0.0453	0.0009
	N	14	14	13
Month 5 2009 & 2010	Mean	244.9	147.9	141.9
	Range	148-351	70-223	92-179
	P-value	—	0.1765	0.1940
	N	7	7	7
Month 7 2010	Mean	540.5	317.3	257.3
	Range	275-930	157-544	175-318
	P-value	—	0.2359	0.1677
	N	4	4	4

[1] P-values indicating significant differences with controls are underlined.

Compositional Analysis of the H Variety and Event H37

The composition data for H37 represent the summary from 6 replicated field trials conducted in 2010. . In addition some acylamide data were collected and summarized in 2009 and 2011. Tubers of event H37 were equivalent to those of the untransformed H variety except for the following:

- H37 tubers contained, on average, 877.5 parts per million (ppm) free ASN compared to the control, which contained 2805 ppm (APV <0.0001). This change was expected and is linked to a reduced acrylamide-forming potential.

- H37 tubers contained, on average, 2724 ppm free GLN compared to the control, which contained 1680 ppm (APV = 0.014). The increase in this amide amino acid counter-balances, in part, losses in the ASN amide amino acid. The new levels are normal for potato.

- H37 tubers contained, on average, 2860 ppm total ASN + ASP compared to the control, which contained 4748 ppm (APV <0.0001). The new level fits within the tolerance interval and is still normal for potato.

- H37 tubers contained, on average, 5159 ppm total GLN + GLU compared to the control, which contained 3926 ppm (APV = 0.05). The new levels are normal for potato.

- Potato chips from H37 potato tubers contained 223.0 ppb acrylamide compared to the control, which had 531.2 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in free ASN and GLN, along with total ASN + ASP, and total GLN + GLU because as non-essential amino acids they can be synthesized by the body. However, the reduced potential of H37 to form acrylamide would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

The following changes were *observed*:

- H37 tubers contained, on average, 207 ppm free LYS as compared to controls, which contained 263 ppm (APV = 0.035). The new levels are still normal for potato.

- H37 tubers contained, on average, 0.13% of sucrose as compared to controls, which contained 0.11% (P-value = 0.0450). The increase in sucrose is often related to a decrease in reducing sugars, which was expected from silencing the promoters associated with the *R1* and *PhL* genes, but was not statistically different from controls. The new levels of sucrose are still normal for potato.

It can be concluded that tubers of event H37 are substantially equivalent to untransformed H tubers except for: (1) changes in ASN and GLN, (2) lower levels of total ASP + ASN, (3) higher levels of total GLN + GLU, (4) slightly decreased amounts of free LYS, (5) slightly increased levels of sucrose, and (6) reduced

acrylamide as expected and intended. In all cases, these compositional changes fall within the normal range for potatoes.

Compositional Analysis of the H Variety and Event H50

The composition data for H50 represent the summary from 6 replicated field trials conducted in 2010. In addition some acylamide data were collected and summarized in 2009 and 2011. Tubers of event H50 were equivalent to those of the untransformed H variety except for the following expected changes:

- H50 tubers contained, on average, 673 parts per million (ppm) free ASN compared to the control, which contained 2805 ppm (APV <0.0001). This change was expected and is linked to a reduced acrylamide-forming potential.

- H50 tubers contained, on average, 2913 ppm free GLN compared to the control, which contained 1680 ppm (APV = 0.006). The increase in this amide amino acid counter-balances, in part, losses in the ASN amide amino acid. The new levels are normal for potato.

- H50 tubers contained, on average, 2655 ppm total ASN+ASP compared to the control, which contained 4748 ppm (APV <0.0001). The new level fits within the tolerance interval and is still normal for potato.

- H50 tubers contained, on average, 5443 ppm GLN+GLU compared to the control, which contained 3926 ppm (APV = 0.018). The new levels are normal for potato.

- Potato chips from H50 potato tubers contained 174.7 ppb acrylamide compared to the control, which had 531.2 ppb (P-value <0.0001). This change was intended.

- The impact on nutritional quality would be minimal from the observed differences in free ASN and GLN, along with total ASN + ASP, and total GLN + GLU because as non-essential amino acids they can be synthesized by the body. However, the reduced potential of H50 to form acrylamide would benefit consumers by addressing a potentially critical food safety issue for the potato industry, particularly in light of recent toxicology studies (NTP 2012).

It can be concluded that tubers of event H50 are substantially equivalent to untransformed H tubers except for: (1) changes in ASN and GLN, (2) lower levels of total ASP + ASN, (3) higher levels of total GLN + GLU, and (4) reduced acrylamide when compared to controls as expected and intended. In all cases, these compositional changes fall within the normal range for potatoes.

Storage Studies: Reducing Sugars and Acrylamide

The objective of the composition studies was to compare all events with their respective controls mostly at the time of harvest. In addition, since many potatoes are stored for months before processing into fries or chips, we tested some of the potatoes for two important attributes, reducing sugars and acrylamide, following storage.

Reducing Sugars. As was noted in the **Results** section (**Tables 5, 11, 17, 23, and 29)**, many of the events had lowered levels of reducing sugars either at the time of harvest or after storage for 1 month (F10, F37, E12, E24, J3, and J55, J78). However, in most cases we did not observe significant differences after 2-5 months of storage. This led us to conclude that silencing of the promoters associated with the *PhL/R1* genes effectively lowered reducing sugars near the time of harvest but these differences were not sustained throughout storage for 2-5 months.

Acrylamide. Samples of fries or potato chips were tested for up to 7 months after harvesting, depending upon the variety. The Atlantic controls and events J3, J55, and J78 were tested only through 2 months of storage because typically that variety would not be stored. Throughout storage, acrylamide levels were consistently lower ($p < .0001$) in the events compared with controls (**Tables 6, 12, 18, 24, and 30**), although there were exceptions with the G and H varieties. In event G11, we observed a mean value of 2,050 ppb compared with 1,769 ppb for the control after 1 month of storage. This was unusual and throughout the rest of the study, acrylamide levels in event G11 were always much lower than controls (46 to 58% reduction at months 5 and 7). For variety H, acrylamide levels were always lower in events H37 or H50, but not statistically significant in months 5 and 7. Overall, the modifications from insertion of pSIM1278 resulted in dramatic reductions in acrylamide that persisted throughout the typical storage periods.

Glycoalkaloids

Glycoalkaloids are toxins commonly found in Solanaceous crops, including potato. The principle glycoalkaloids in potatoes are α-solanine and α-chaconine. For food safety purposes, an upper limit for glycoalkaloid content of 20 mg per 100 g of potato is generally accepted (Sinden 1987; Crocco 1981). This research confirmed that tubers of the events contained equivalent levels of natural glycoalkaloids compared with untransformed controls.

For the varieties included here, most values for glycoalkaloids were close to or below the accepted level of 20 mg per 100 g of potato (**Table 31**). Both untransformed controls and events for the G variety had mean values that were about the level of 20 mg/100 g. This has been attributed to excessive handling at storage facilities, resulting in increased exposure to light, and thus, higher levels of glycoalkaloids (Percival 1999). Although the control mean values nearly exceeded the accepted levels, the G variety has a long history of safe use and the slightly higher than expected results would not be expected in normal commercial operations.

Also, the range of values for E24 exceeded 20 mg/100 g but the mean was less than and not significantly different from the Russet Burbank control. Although a cause was not identified, the higher value for E24

could be from excessive exposure to light (Percival 1999). In all cases, the events were equivalent to the controls with no observed significant differences. All glycoalkaloid levels were within the normal ranges for potato, when compared with the tolerance intervals or combined literature ranges.

Table 31: Glycoalkaloids (mg/100 g)[1] in Ranger Russet, Russet Burbank, Atlantic, G, and H Varieties.

Variety	Total Glycoalkaloids (mg/100 g)				
	Mean	Range	P-Value	TI	CLR[2]
Ranger Control	6.704	3.06-10.23		0.000-33.05	3.20-210.4
F10	6.979	2.84-9.51	0.8285		
F37	6.805	4.28-9.42	0.9812		
Burbank Control	7.404	2.74-18.59		0.000-33.05	3.20-210.4
E12	5.678	1.69-10.53	0.1707		
E24	6.845	1.45-33.81	0.8174		
Atlantic Control	6.274	2.09-11.40		0.000-33.05	3.20-210.4
J3	5.723	1.62-11.67	0.2393		
J55	5.864	1.83-11.12	0.4833		
J78	6.084	1.65-14.27	0.7198		
G Control	19.82	6.17-44.40		0.000-33.05	3.20-210.4
G11	20.32	5.81-55.3	0.9153		
H Control	10.65	5.96-21.07		0.000-33.05	3.20-210.4
H37	10.76	5.41-17.25	0.8951		
H50	11.30	6.23-22.81	0.4379		

[1]TI = Tolerance Interval, CLR = Combined Literature Range. P-values indicating significant differences with controls are underlined.
[2]Literature ranges from Kozukue *et al.* (2008).

MATERIALS AND METHODS

Field Trial Locations. All locations and varieties have been summarized in **Table 32**. Each combination of trial site, event or contol, and replication would represent one sample for the compositional assessment. Some trials were conducted only for agronomic and phenotypic data as indicated in **Table 32**, and in addition most were used for full composition although some were only tested for acrylamide and sugars. **32**

Table 32: Field Trial Locations

USDA Notification #	Site State	Site County	Material Tested	Trial Design[3]	Rows x Planted Tubers/ Rep	Seed Type	Regional Specifics	Location ID Code	Composition Data
2009 Field trials									
09-049-114n	ID	Canyon	Ranger Russet, Russet Burbank, Atlantic	RCB, 5 reps/line (1 rep/line for Atlantic)	1x20 (1x15 for Atlantic)	Mini-tubers	Typical for Southwest Idaho, an important potato-growing region in a state in the Northwest that produces about 120 million cwt/year, mainly for the French fry industry, Careful management is needed to limit or prevent heat-associated agronomic issues.	Not required[1]	Ranger Russet, Russet Burbank, only
09-049-114n	ID	Bingham	Ranger Russet, Russet Burbank	RCB, 5 reps/line	1x20	Mini-tubers	Typical for Southeast Idaho, an important potato-growing region in a state that produces about 120 million cwt/year, mainly for the French fry industry, with harvests in Fall. The growing season is relatively short because of cooler temperatures.	Not required[1]	Ranger Russet, Russet Burbank
09-049-114n	WI	Oneida	G, H	1 rep/line	1x100	Mini-tubers	Typical for Wisconsin, a Midwest state that produces about 20 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall. Large areas are dominated by muck soils.	Not required[1]	Agronomic and phenotypic data only
08-353-103n	FL	St. Johns	G, H	RCB, 4 reps/line	1x25	Mini-tubers	Typical for Florida that produces almost 8 million cwt/year, mainly for the chip and fresh potato industry, with harvests in Spring.	Not required[1]	Agronomic and phenotypic data only
08-353-104n	MI	Montcalm	Ranger Russet, Russet Burbank, G, H	RCB, 4 reps/line	1x20	Mini-tubers	Typical for Michigan, a Midwest state that produces about 15 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall. The climate is characterized by mild temperatures and ample rain.	Not required[1]	Ranger Russet, Russet Burbank – complete composition G, H – Acrylamide

Table 32 (Continued): Field Trial Locations

USDA Notification #	Site State	Site County	Material Tested	Trial Design[3]	Rows x Planted Tubers/ Rep	Seed Type	Regional Specifics	Location ID Code	Composition Data
2010 Field trials									
10-053-132n	ID	Canyon	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line	3x20	Field seed (G1)	*See above for Canyon county*	UOIPAR2010	Ranger Russet, Russet Burbank, Atlantic
10-053-132n	ID	Bingham	Ranger Russet, Russet Burbank, Atlantic, G, H	RCB, 3 reps/line (4 reps/line for G, H; 5 reps/line for Atlantic)	3x20 (1x20 for G, H, Atlantic)	Field seed (G1) (Mini-tubers for Atlantic)	*See above for Bingham county*	UOIABE2010	Ranger Russet, Russet Burbank, Atlantic, G, H
10-053-132n	WI	Oneida	G, H	RCB, 4 reps/line	1x20	Field seed (G1)	*See above for Oneida county*	RHI2010	G, H
10-053-132n	WI	Adams	Atlantic	RCB, 5 reps/line	1x20	Mini-tubers	Typical for Wisconsin, a Midwest state that produces about 20 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall.	WI2010	Atlantic
10-076-013n	WI	Vilas	G,H	1 rep	Variable[4]	Mini-tuber/ G1[5]	Typical for Wisconsin, a Midwest state that produces about 20 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall.	VILAS2010	Seed production only
10-053-132n	MI	Montcalm	Atlantic, G, H	RCB, 3 reps/line (4 reps/line for G, H)	3x20 (1x20 for G, H)	Mini-tubers	*See above for Montcalm county*	MSUMONT2010	Atlantic, G, H
10-053-132n	MI	Missaukee	G, H	RCB, 4 reps/line	1x20	Field seed (G1)	Typical for Michigan, a Midwest state that produces about 15 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall.	MSULAKE2010	G, H
10-053-132n	NE	Cherry	Atlantic	RCB, 5 reps/line	1x20	Field seed (G1) (Mini-tubers for Atlantic)	Typical for Nebraska, a Midwest state that produces about 9 million cwt/year, mainly for the seed industry, with harvests in Fall.	CSS2010	Atlantic
10-053-132n	ND	Grand Forks[2]	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line (5 reps/line for Atlantic)	3x20 (1x20 for Atlantic)	Field seed (G1) (Mini-tubers for Atlantic)	Typical for North Dakota, a Midwest state that produces about 20 million cwt/year, mainly for the French fry industry, with harvests in Fall. The Red River Valley is the 3rd largest potato growing region in the US.	NDSU2010	Ranger Russet, Russet Burbank, Atlantic
10-053-132n	WA	Adams	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line (5 reps/line for Atlantic)	3x20 (1x20 for Atlantic)	Field seed (G1) (Mini-tubers for Atlantic)	Typical for Washington, a state in the Northwest that produces about 85 million cwt/year, mainly for the French fry industry, with harvests in Fall. Ideal growing conditions give rise to very high yields per acre.	WSU2010	Ranger Russet, Russet Burbank, Atlantic
09-336-103n	FL	St. Johns	G, H	RCB, 4 reps/line	1x25	Field seed (G1)	*See above for St. Johns county*	UFL2010	G, H

Table 32 (Continued): Field Trial Locations

USDA Notification #	Site State	Site County	Material Tested	Trial Design[3]	Rows x Planted Tubers/ Rep	Seed Type	Regional Specifics	Location ID Code	Composition Data
2011 Field trials									
11-063-103n	ID	Canyon	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line	3x20	Field seed (G2) (G1 for Atlantic, Mini-tubers for F37)	*See above for Canyon county*	UOIPAR2011	Ranger Russet, Russet Burbank, Atlantic
11-063-103n	ID	Bingham	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line	3x20	Field seed (G2) (G1 for Atlantic, Mini-tubers for F37)	*See above for Bingham county*	UOIABE2011	Ranger Russet, Russet Burbank, Atlantic
11-063-103n	WI	Adams	Atlantic	RCB, 3 reps/line	3x20	Field seed (G1)	*See above for Adams county, Wisconsin*	WI2011	Atlantic
11-063-103n	MI	Montcalm	Atlantic	RCB, 3 reps/line	3x20	Field seed (G1)	*See above for Montcalm county*	MSUMONT2011	Atlantic
11-063-103n	ND	Grand Forks	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line	3x20	Field seed (G2) (G1 for Atlantic, Mini-tubers for F37)	*See above for Grand Forks county*	NDSU2011	Ranger Russet, Russet Burbank, Atlantic
11-063-103n	WA	Adams	Ranger Russet, Russet Burbank, Atlantic	RCB, 3 reps/line	3x20	Field seed (G2) (G1 for Atlantic, Mini-tubers for F37)	*See above for Adams county, Washington*	WSU2011	Ranger Russet, Russet Burbank, Atlantic
10-326-103n	FL	St. Johns	G, H, Atlantic	RCB, 4 reps/line	1x20	Field seed (G1)	*See above for St. Johns county*	UFL2011	Atlantic – complete composition G, H – Acrylamide
11-063-103n	WI	Oneida	G, H	RCB, 4 reps/line	1x20	Field seed (G1)	*See above for Oneida County*	RHI2011	G, H - Acrylamide
11-063-103n	IN	Pulaski	Atlantic	RCB, 3 reps/line	1x20	Field seed (G1)	Typical for Indiana, a Midwest state that produces about 2 million cwt/year, for both the chip and fresh potato industry, with harvests in Fall.	INDIANA2011	Atlantic
11-094-106n	WI	Oneida	G,H	RCB, 4 reps/ line	1x13	Field seed (GI)	*See above for Oneida County*	ONEIDA2011	G - Acrylamide

[1]USDA/APHIS did not require a unique location identification code in 2009.

[2]Because parts of this field were flooded extensively, Ranger Russet and Russet Burbank tubers were evaluated only biochemically.

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

[4]Several lines were grown

[5]Field grown/mini-tuber

Testing Laboratories. Analytical testing for Ranger Russet and Russet Burbank samples were completed partially by the J.R. Simplot Co., Boise, ID in 2009 and 2010, with the remainder done by Covance Laboratories, Inc., Madison, WI. Atlantic samples were analyzed partially by Simplot and partially by Covance in 2010. In 2011, all analyses were completed at Covance Labs. All testing for G and H varieties was conducted by Covance Labs. Clarification of testing location by analysis is provided in **Table 33**. .In 2011 we made a decision to only use one labarotory for compositional data analysis for regulatory data. Altough data were collected in two laboratories, both used standard procedures for calibrating their instruments and were treated similarly in the statistical analysis.

Table 33: Laboratory Facilities Used for Compositional Analysis

Analysis	2009		2010		2011	
	Ranger Russet, Russet Burbank	G and H[1]	Ranger Russet, Russet Burbank, Atlantic	G and H	Ranger Russet, Russet Burbank, Atlantic	G and H[1]
Key Proximates, Vitamins and Minerals	Covance	NA	Covance	Covance	Covance	NA
Free Amino Acids	Simplot	NA	Simplot	Covance	Covance	NA
Total Amino Acids	Simplot	NA	Simplot	Covance	Covance	NA
Sugars	Simplot	NA	Simplot	Covance	Covance	NA
Acrylamide	Covance	Covance	Covance	Covance	Covance	Covance
Glycoalkaloids	Simplot	NA	Simplot	Covance	Covance	NA
Vitamin C	Simplot	NA	Simplot	Covance	Covance	NA

[1]Compositional data not completed for G and H in 2009 and 2011, except for acrylamide

Sample preparation. For the Ranger Russet, Russet Burbank, and Atlantic varieties, samples of each event and controls were obtained by randomly selecting 5 tubers of about 6 inches in length from each rep and site. Three to nine longitudinal slices with skin from each tuber were taken from the top, bottom, and center. 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 Ranger Russet and Russet Burbank potatoes were processed into french fries, using standard practice for the foodservice french fry industry. The Atlantic variety and events were processed using standard practice for potato chips.

For the G variety, event G11, the H variety and events H37 and H50, two tubers per replication, 2" to 3" in diameter, were picked from each of 4 replications from 6 locations. All compositional analyses for G and H varieties were performed at Covance Labs according to standard industry protocols. Fresh tubers were used for all analyses except for acrylamide. Samples for acrylamide were prepared by slicing 8 tubers per replication and combining 2 slices per tuber for a total of 16 slices and then frying in a batch fryer, for the purpose of making potato chips.

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 lyophilization. The equation used for calculation was:

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

Moisture content: Covance Labs. Moisture levels were determined by Covance Laboratories using Covance protocol M100T100_S:4 (AOAC 2005c; AOAC 2005d).

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

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 (AOAC 2005f).

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) (AOAC 2005g; AOAC 2005h).

Fat: Covance Labs. Fat was determined by Acid Hydrolysis using Covance protocol FAAH_S:7(AOAC 2005i; AOAC 2005j).

Niacin: Covance Labs. Niacin was determined by Covance Laboratories using Covance protocol NIAP_S:11 **(**AOAC 2005k; AOAC 2005l).

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 (AOAC 2005m; AOAC 2005n).

Pyridoxine: Pyridoxine was determined by Covance Laboratories using Covance protocol B6A_S:11 (AOAC 2005o, 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. In brief, freeze-dried tuber powder (about 200 mg) was placed into a 2 mL screw cap tube along 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 (AOAC 2005b).

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 resuspended 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 0.1 mg/g dry weight (DW) (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 (Schuster 1988; Henderson 2000; and Barkholt and Jensen 1989).

Tryptophan: Covance Labs. Tryptophan levels were determined by Covance Laboratories using the Covance protocol TRPLC_S:3 (AOAC 2005a).

Total amino acids: Simplot Lab. Amino acid hydrolysis for total amino acid analysis was performed as previously described (Purcell *et al*. 1972; Miedel *et al.* 1989) with optimization. About 50 mg freeze dried tissue was weighed and transferred into the bottom of a vacuum hydrolysis tube (5 mL, 10 mm 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 ASNs 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 (Schuster 1988; Henderson 2000; and Barkholt and Jensen 1989).

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 for the Russet Burbank, Ranger Russet, and Atlantic lines were analyzed by combining data from multiple test years and locations using the following linear mixed model:

$$Y_{ijkm} = \alpha_{i+} \beta_j + \gamma_{k(j)} + \delta_{l(j,k)} + (\alpha\gamma)_{ik} + \varepsilon_{ijklm}$$

- α = mean of treatment (fixed)
- β = year (random)
- γ = effect of location [year] (random)
- δ = rep[year, location] (random)
- ε = residual random error

$\beta_j \sim iidN(0, \sigma^2_{year})$, $\gamma_{k(j)} \sim iidN(0, \sigma^2_{loc(year)})$, $\delta_{l(j,k)} \sim iidN(0, \sigma^2_{rep(year, loc)})$, $(\alpha\gamma)_{ik} \sim iidN(0, \sigma^2_{loc*treatment})$, and $\varepsilon_{ijkm} \sim iidN(0, \sigma^2)$

(This means that all error terms are assumed to be normally distributed with a mean of 0 and equal variances).

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), $\gamma_{k(j)}$ 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.

We tested all data for normality before conducting the analysis mentioned here. Most data met the requirement, but with some attributes such as free amino acids, many distributions were skewed by being too close to the detection limit. In those cases, we simply reported means and ranges and did not test for statistical significance.

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:

$$Y_{ijkm} = \alpha_{i+}\beta_j + \gamma_{k(j)} + (\alpha\beta)_{ij} + \varepsilon_{ijkm}$$

- α = mean of treatment (fixed effect)
- β = effect of location (random)
- γ = rep within location effect (random)
- ε = residual random error

$\beta_j \sim iidN(0,\sigma^2_{loc})$, $\gamma_{k(i)} \sim iidN(0, \sigma^2_{loc(rep)})$, $(\alpha\beta)_{ij} \sim iidN(0, \sigma^2_{loc*treatment})$, and $\varepsilon_{ijkm} \sim iidN(0, \sigma^2)$

(This means that all error terms are assumed to be normally distributed with a mean of 0 and equal variances).

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_{ijkm} denotes the residual random error.

Following analysis with the mixed linear models, we calculated the False Discovery Rate to minimize the chance of mistakenly concluding that the transformed events were different from controls. When applying this technique, we organized the data into logical related groups for comparison. Those groups

were: composition, free amino acids, and total amino acids. Thus, the difference between least squares (LS) means of treatment lines and the LS means of corresponding control lines for traits under compositional, free amino acids and total amino acids groups was first compared using Students pair-wise comparison method. Then, the P-values were adjusted following the false discovery rate (FDR) approach (Benjamini and Hochberg 1995) within each group, to control the percentage of false signals. A significant difference was established with a FDR-Adjusted P-Value $\leq$ 0.05.

For traits considered independent of the basic composition, it would not be desirable to apply the FDR method. For example, potato yield, acrylamide, sugar, and glycoalkaloids would not be closely related to basic composition, so it would be preferred to use only Tukey's test to compare the events with controls. Thus, the difference between LS means of treatment lines and the LS means of corresponding control lines for yield, acrylamide, sugar and glycoalkaloids was compared using Tukey's method. 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): Snowden, Chieftain, Red Norland, Ida Rose, Russet Burbank, Ranger Russet, Atlantic, G, and H. Total data points were 317 for proximate analysis, 193 for glycoalkaloids, 337 for both free amino acids, 252 total amino acids, and 227 for sugars. The tolerance intervals were calculated to contain, with 95% confidence, 99% of the values contained in the population. This statistical 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.

CONCLUSIONS

The compositional assessment studies, evaluating proximates, vitamins, amino acids, minerals, sugars, and glycoalkaloids, were conducted on Innate™ potatoes to 1) show equivalence to the untransformed controls, 2) compare the Innate™ potatoes to literature ranges, where applicable; and 3) show that there were no changes in allergens or potential toxins. Ultimately, results of the composition studies showed food safety equivalence between the events and the untransformed controls.

Results of all significant differences between the events and controls are summarized in **Table 34** for Ranger Russet and Russet Burbank and in **Table 35** for Atlantic, G, and H varieties. Most of these differences; reduced free ASN and total ASP + ASN, increased free GLN and total GLU + GLN, reduced acrylamide, and lowered reducing sugars were expected because of the intended gene silencing. Other significant differences included decreased free VAL (J3, J55), decreased free LYS (H37), increased free ARG (F37), increased total LYS (G11), increased total PRO (G11), increased Vitamin C (F10, F37), increased niacin (F10, F37), decreased pyridoxine (J3, J55), decreased sucrose (E12, E24), and increased sucrose (H37). All these observed differences fell within the tolerance intervals or combined literature values, and therefore were considered to be within normal ranges for potatoes.

It's important to note that for events E12 and E24, the free ASN levels were not statistically different from controls at the adjusted $p \leq .05$ level. However, these events showed a 74 to 76% reduction in free ASN, which was significant at the adjusted $p = .11$ level. Such large differences were sometimes not statistically significant, which was a direct result of using the correction for False Discovery Rate (Benjamini and Hochberg 1995). These reductions were of practical significance and are considered similarly to the other events regarding this trait.

Note that free amino acids would not normally be part of a compositional assessment, however, they were included because the quantity of free asparagine is important to show the efficacy of silencing the *Asn1* gene for the purpose of reducing acrylamide. Many of the free amino acids are found at such low levels in potatoes that values barely exceed the detection limit. We chose to adjust all values that were below detection limits to 100 to complete the statistical analysis, and have reported the mean values. However, many of these values were too low to allow an estimate of variance, so P-Values were not reported. It might be just as appropriate to report most of these values as "Not Available", but we chose to include them. For the purpose of assessing the nutritional equivalence of the events, we recommend that the focus should be on comparing total amino acids to controls.

The most important change between tubers of the events and their untransformed controls relates to reduction of the amino acid asparagine (ASN) and reducing sugars. Reduced amounts of free ASN and reducing sugars in potatoes of the events resulted in lowered acrylamide after heat processing compared to the conventional potatoes.

Table 34. Statistical Differences Observed in Composition Data: Events F10, F37, E12 and E24

Variety	Event	Attribute	Difference Expected?	Comments
Ranger Russet	F10	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Increased Niacin (B3)	No	Within TI and CLR
		Increased Vitamin C	No	Within TI and CLR
Ranger Russet	F37	Decreased Free ASN	Yes	
		Increased free ASP	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Increased Vitamin C	No	Within TI and CLR
		Increased Niacin (B3)	No	Within TI and CLR
		Increased free ARG	No	Within TI and CLR
Russet Burbank	E12	Decreased Free ASN	Yes	74% reduced, not significant
		Decreased Total ASN+ASP	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Sucrose	No	Within TI and CLR
Russet Burbank	E24	Decreased Free ASN	Yes	76% reduced, not significant
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Sucrose	No	Within TI and CLR

TI = tolerance interval
CLR = combined literature range

Table 35. Statistical Differences Observed in Composition Data: Events J3, J55, J78, G11, H37 and H50

Variety	Event	Attribute	Difference Expected?	Comments
Atlantic	J3	Decreased Free ASN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Pyridoxine (B_6)	No	Within TI and CLR
		Decreased Free VAL	No	Within TI and CLR
Atlantic	J55	Decreased Free ASN	Yes	
		Decreased Reducing Sugars	Yes	Glucose and Fructose
		Reduced Acrylamide	Yes	
		Decreased Pyridoxine (B_6)	No	Within TI and CLR
		Decreased Free VAL	No	Within TI and CLR
Atlantic	J78	Decreased Free ASN	Yes	Adjusted P = .0548
		Decreased Reducing Sugars	No	Glucose and Fructose
		Reduced Acrylamide	Yes	
Variety G	G11	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Reduced Acrylamide	Yes	
		Increased Total LYS	No	Within TI and CLR
		Increased Total PRO	No	Within TI and CLR
Variety H	H37	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Increased Sucrose	No	Within TI and CLR
		Reduced Acrylamide	Yes	
		Decreased Free LYS	No	Within TI and CLR
Variety H	H50	Decreased Free ASN	Yes	
		Increased Free GLN	Yes	
		Decreased Total ASP+ASN	Yes	
		Increased Total GLU+GLN	Yes	
		Reduced Acrylamide	Yes	

TI = tolerance interval
CLR = combined literature range

For the compositional assessment, all events were compared with their respective controls mostly at the time of harvest. In addition, the potatoes were tested for reducing sugars and acrylamide, following storage.

Many of the events had lowered levels of reducing sugars either at the time of harvest or after storage for 1 month (F10, F37, E12, E24, J3, and J55, J78), however, in most cases we did not observe significant differences after 2-5 months of storage. It was not expected that J78 would have lowered levels of reducing sugars because it was one of two events, J78 and G11, that did not contain the R1/PhL promoter silencing cassette. Tubers of events G11, H37, and H50 contain the same amount of reducing sugars as tubers of their untransformed counterparts. The inability of the silencing construct to limit glucose/fructose formation in H37 and H50 may be due to the fact that the H variety is naturally low in glucose and fructose. Thus, we concluded that silencing of the promoters associated with the *PhL/R1* genes effectively lowered reducing sugars near the time of harvest in most events but these differences were not sustained throughout storage for 2-5 months.

During storage of up to 7 months, acrylamide levels in most events were consistently lower ($p < .0001$) than controls, although there were exceptions with the G and H varieties. In event G11, we observed a mean of 2,050 ppb compared with 1,769 ppb for the control after 1 month of storage. This was unusual and throughout the rest of the study, acrylamide levels in event G11 were always much lower than controls (46 to 58% reduction at months 5 and 7). For variety H, acrylamide levels were always lower in events H37 or H50, but not statistically significant in months 5 and 7. Overall, the modifications from insertion of pSIM1278 resulted in dramatic reductions in acrylamide that persisted throughout the typical storage periods.

This research also confirmed that the events are as safe as controls and did not contain increased levels of natural glycoalkaloids, which are toxins commonly found in Solanaceous crops, including potato. Based on the lack of differences between the events and controls, there would not be a safety issue as a result of the introduction of the reduced acrylamide potential and low black spot bruise traits in Innate™ potatoes. All glycoalkaloid levels were within the normal ranges for potato, when compared with the tolerance intervals or combined literature ranges.

Because tubers of the events are substantially equivalent to tubers from the untransformed controls, except for the intended result of lowered ASN, reducing sugars, and acrylamide, the potatoes will be as safe as their untransformed controls for use as food and feed. In contrast, the transition from standard tubers to Innate™ tubers, which have significantly lower acrylamide-forming potential, will reduce the average total exposure to dietary acrylamide by almost one-third because about 42% of this acrylamide is associated with the consumption of french fries and potato chips in the United States and Europe (Dybing *et al*. 2005; Mucci and Wilson 2008).

While some statistically significant differences in analyte levels were found and discussed, in cases where the Innate™ tubers were statistically different from the untransformed control variety, the values remained within the natural variation for potatoes, based on tolerance intervals calculated from the controls or literature references.

REFERENCES

AOAC (2005a) Official Methods of Analysis of AOAC International, 18th Ed., Method 988.15. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005b) Official Methods of Analysis of AOAC International, 18th Ed., Method 967.22. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005c) Official Methods of Analysis of AOAC International, 18th Ed., Method 925.09. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005d) Official Methods of Analysis of AOAC International, 18th Ed., Method 926.08. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005e) Official Methods of Analysis of AOAC International, 18th Ed., Method 923.03. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005f) Official Methods of Analysis of AOAC International, 18th Ed., Method 962.09. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005g) Official Methods of Analysis of AOAC International, 18th Ed., Method 984.27. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005h) Official Methods of Analysis of AOAC International, 18th Ed., Method 985.01. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005i) Official Methods of Analysis of AOAC International, 18th Ed., Method 922.06. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005j) Official Methods of Analysis of AOAC International, 18th Ed., Method 954.02. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005k) Official Methods of Analysis of AOAC International, 18th Ed., Method 944.13. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005l) Official Methods of Analysis of AOAC International, 18th Ed., Method 960.46. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005m) Official Methods of Analysis of AOAC International, 18th Ed., Method 955.04. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005n) Official Methods of Analysis of AOAC International, 18th Ed., Method 979.09. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2005o) Official Methods of Analysis of AOAC International, 18th Ed., Method 961.15.Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

AOAC (2006) Official Methods of Analysis of the AOAC International, 19th Edition, Method 997.13. Association of Official Agricultural Chemists International, Gaithersburg, MD, USA.

Atkin L, Schultz AS, Williams WL, Frey CN (1943) Yeast micro- biological methods for determination of vitamins. Industrial and Eng Chem, Analytical Ed, 15: 141-144.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B (Methodological), 57: 289–300.

Barkholt V, Jensen AL (1989) Amino acid analysis: determination of cystine plus half-cystine in proteins after hydrochloric acid hydrolysis with a disulfide compound as additive. Analytical Biochem, 177: 318-322.

Blenkinsop RW, Copp LJ, Yada RY, Marangoni A (2002) Changes in compositional parameters of tubers of potato (*Solanum tuberosum*) during low-temperature storage and their relationship to chip processing quality. J Agric Food Chem, 50: 4545-4553.

Crocco S. *Potato sprouts and greening potatoes: Potential toxic reaction*. United Stated Food and Drug Administration, Poisonous Plant Database (1981). Accessed Online: 12 May 2011.

Davies AMC (1977) The free amino acids of tubers of potato varieties grown in England and Ireland. Potato Res, 20: 9-21.

Dybing E, Farmer PB, Andersen M, Fennell TR, Lalljie SP, Müller DJ, Olin S, Petersen BJ, Schlatter J, Scholz G, Scimeca JA, Slimani N, Törnqvist M, Tuijtelaars S, Verger P (2005) Human exposure and internal dose assessments of acrylamide in food. Food Chem Toxicol, 43: 365-410.

FDA (2003) United States Food and Drug Administration, Center for Food Safety and Applied Nutrition of Plant & Dairy Foods and Beverages. *Detection and Quantification of Acrylamide in Foods*. FDA, 2003. Accessed Online: 23 May 2011.

Henderson JW, Ricker RD, Bidlingmeyer BA, Woodward C (2000) Rapid, accurate, sensitive, and reproducible HPLC analysis of amino acids, amino acid analysis using Zorbax Eclipse-AAA columns and the Agilent 1100 HPLC. Agilent Publication.

Horton DE, Anderson JL (1992) Potato production in the context of the world and farm economy. In: The potato crop: the scientific basis for improvement (Ed. Harris PM) Chapman and Hall, London, pp. 803.

Kozukue N, Yoon KS, Byun GI, Misoo S, Levin CE, Friedman M (2008) Distribution of glycoalkaloids in potato tubers of 59 accessions of two wild and five cultivated Solanum species. J Agric Food Chem, 56: 11920-11928.

Lilla Z, Sullivan D, Ellefson W, Welton K, Crowley R (2005) Determination of “net carbohydrates” using high-performance anion exchange chromatography. J of AOAC International, 88: 714- 719.

Lisinska G, Leszczynski W (1989) Potato science and technology. Chapter 2: Potato tubers as a raw material for processing and nutrition. Elsevier Applied Science, London, pp 11-128.

Matsuura-Endo CM, Ohara-Takada A, Chuda Y, Ono H, Yada H, Yoshida M, Kobayashi A, Tsuda S, Takigawa S, Noda T, Yamauchi H, Mori M (2006) Effects of storage temperature on the contents of sugars and free amino acids in tubers from different potato cultivars and acrylamide in chips. Biosci Biotechnol Biochem, 70: 1173-1180.

Menendez CM, Ritter E, Schafer-Pregl R, Walkemeier B, Kalde A, Salamini F, Gebhardt C (2002) Cold sweetening in diploid potato: mapping quantitative loci and candidate genes. Genetics, 162: 1423-1434.

Miedel MC, Hulmes JD, Pan YCE (1989) The use of fluorescamine as a detection reagent in protein microcharacterization. J Biochem Biophys Methods, 18: 37-52.

Mucci LA, Wilson KM (2008) Acrylamide intake through diet and human cancer risk. J Agric Food Chem, 56: 6013-6019.

NTP (2012) Technical Report on the Toxicology and Carcinogenesis Studies of Acrylamide in F344/N Rats and B6C3F1 Mice. National Toxicology Program. National Institutes of Health. Public Health Service. U.S. Dept. of Health and Human Services.

Percival G (1999) Light-induced glycoalkaloid accumulation of potato tubers (*Solanum tuberosum L*). J Sci Food Agric, 79: 1305-1310.

Purcell A, Swaisgood HE, Pope DT Jr, (1972) Protein and amino acid content of sweetpotato cultivars. Am Soc Hortic Sci, 97: 30.

Rogan GJ, Bookout, JT, Duncan DR, Fuchs RL, Lavrik PB, Love SL, Mueth M, Olson T, Owens ED, Raymond PJ, Zalewski J (2000) Compositional analysis of tubers from insect and virus resistant potato plants. J Agric Food Chem, 48: 5936-5945.

Schuster R (1988) Determination of amino acids in biological, pharmaceutical, plant and food samples by automated precolumn derivitization and HPLC. J Chromatogr, 431: 271-284.

Shakya R, Navarre DA (2006) Rapid screening of ascorbic acid, glycoalkaloids and phenolics in potato using high-performance liquid chromatography. J of Ag and Food Chem, 54: 5253-5260.

Shepherd LVT, Bradshaw JE, Dale MFB, McNicol JW, Pont SDA, Mottram DS, Davies HV (2010) Variation in acrylamide producing potential in potato: Segregation of the trait in a breeding population. Food Chem, 123: 568-573.

Sinden SL (1987) Potato glycoalkaloids. Acta Hort, 207: 41-48.

Talburt WF, Smith O (1987) Potato processing. Van Nostrand Reinhold, New York.

Talley EA, Toma RB, Orr PH (1984) Amino acid composition of freshly harvested and stored potatoes. Am Pot Journal, 61: 267-279.

USDA (1973) United States Department of Agriculture, Energy Value of Foods. Agricultural Handbook, No. 74, pp. 2-11.

USDA (1975) United States Department of Agriculture, Composition of Foods Agricultural Handbook, No. 8, pp. 159-160.

Vardeman SB (1992) What about the other intervals?. The American Statistician, 46: 193-197.

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.

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Annex A

Proximates, Vitamins, Minerals, Free Amino Acids, and Total Amino Acids – Combined Literature Ranges (CLR) Used in Tables

Proximates, Vitamins, Minerals

Substance	Units	Value	Reference	Formula Used[1]	CLR[2] Formula Calculation	End Units
Protein low	%	0.70	Lisinska and Leszczynski 1989	NA	**0.70**	%
Protein high	%	4.60	Lisinska and Leszczynski 1989	NA	**4.60**	%
Fat low	%	0.02	Lisinska and Leszczynski 1989	NA	**0.02**	%
Fat high	%	0.20	Lisinska and Leszczynski 1989	NA	**0.20**	%
Ash low	%	0.44	Lisinska and Leszczynski 1989	NA	**0.44**	%
Ash high	%	1.90	Lisinska and Leszczynski 1989	NA	**1.90**	%
Crude fiber low	%	0.17	Lisinska and Leszczynski 1989	NA	**0.17**	%
Crude fiber high	%	3.50	Lisinska and Leszczynski 1989	NA	**3.50**	%
Carbohydrates low	%	13.30	Talburt and Smith 1987	NA	**13.30**	%
Carbohydrates high	%	30.53	Talburt and Smith 1987	NA	**30.53**	%
Calories low	kcal/100g	70.00	Horton and Anderson 1992	NA	**80.00**	kcal/100g
Calories high	kcal/100g dry	551.00	Horton and Anderson 1992	kcal/100g x 0.2[1]	**110.20**	kcal/100g
Moisture low	%	63.20	Talburt and Smith 1987	NA	**63.20**	%
Moisture high	%	86.90	Talburt and Smith 1987	NA	**86.90**	%
B3 low	mg/200g	0.18	Rogan et al. 2000	mg/200g x 0.5mg/100g	**0.09**	mg/100g
B3 high	mg/200g	6.20	Rogan et al. 2000	mg/200g x 0.5mg/100g	**3.10**	mg/100g
B6 low	mg/200g	0.26	Rogan et al. 2000	mg/200g x 0.5mg/100g	**0.13**	mg/100g
B6 high	mg/200g	0.82	Rogan et al. 2000	mg/200g x 0.5mg/100g	**0.41**	mg/100g
Vitamin C low	mg/100g	1.00	Lisinska and Leszczynski 1989	NA	**1.00**	mg/100g
Vitamin C high	mg/100g	54.00	Lisinska and Leszczynski 1989	NA	**54.00**	mg/100g
Cu low	mg/200g	0.03	Rogan et al. 2000	mg/200g x 5 = mg/kg = ppm	**0.15**	ppm
Cu high	mg/200g	1.40	Rogan et al. 2000	mg/200g x 5 = mg/kg = ppm	**7.00**	ppm
Mg low	mg/200g	22.50	Rogan et al. 2000	mg/200g x 5 = mg/kg = ppm	**112.50**	ppm
Mg high	mg/200g	110.00	Rogan et al. 2000	mg/200g x 5 = mg/kg = ppm	**550.00**	ppm
K low	mg/200g	700.00	Rogan et al. 2000	mg/200g x 5 = mg/kg = ppm	**3500.00**	ppm
K high	mg/200g	1250.00	Rogan et al. 2000	mg/200g x 5 = mg/kg = ppm	**6250.00**	ppm

[1]0.2 = approximate conversion from dry weight to fresh weight (internal communication), NA = value already converted
[2]CLR=combined literature range

Free Amino Acids

Substance	Value	Units	Reference	Formula Wt (FW)	Formula Used[1]	CLR[2] (ppm)
ALA low	6.0	mg/100g	Davies 1977	89.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**12**
ALA high	118.0	mg/100g	Davies 1977	89.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**236**
ARG low	60.0	mg/100g	Davies 1977	174.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**120**
ARG high	736.0	mg/100g	Davies 1977	174.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**1472**
ASN low	11.8	mM/kg	Shepherd et al. 2010	132.1	mM/kg x FW x 0.2	**312**
ASN high	3490.0	mg/100g	Davies 1977	132.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**6980**
ASP low	32.0	mg/100g	Davies 1977	133.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**64**
ASP high	376.0	mg/100g	Davies 1977	133.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**752**
GABA low	15.0	mg/100g	Davies 1977	NA	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**30**
GABA high	448.0	mg/100g	Davies 1977	NA	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**896**
GLN low	220.0	mg/100g	Davies 1977	146.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**440**
GLN high	2968.0	mg/100g	Davies 1977	146.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**5396**
GLU low	225.0	mg/100g	Davies 1977	147.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**450**
GLU high	371.0	mg/100g	Davies 1977	147.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**742**
GLY low	1.0	mg/100g	Lisinska and Leszczynski 1989	75.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**2**
GLY high	52.0	mg/100g	Lisinska and Leszczynski 1989	75.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**104**
HIS low	17.0	mg/100g	Davies 1977	155.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**34**
HIS high	328.0	mg/100g	Davies 1977	155.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**656**
ILE low	14.0	mg/100g	Davies 1977	131.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**28**
ILE high	165.0	mg/100g	Davies 1977	131.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**330**
LEU low	8.0	mg/100g	Davies 1977	131.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**16**
LEU high	131.0	mg/100g	Davies 1977	131.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**262**
LYS low	9.0	mg/100g	Davies 1977	146.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**18**
LYS high	319.0	mg/100g	Davies 1977	146.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**638**
MET low	9.0	mg/100g	Davies 1977	149.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**18**
MET high	108.0	mg/100g	Davies 1977	149.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**216**
ORN low	2.0	mg/100g	Davies 1977	NA	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**4**
ORN high	244.0	mg/100g	Davies 1977	NA	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**488**
PHE low	0.0	mg/100g	Davies 1977	165.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**0**
PHE high	204.0	mg/100g	Davies 1977	165.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**408**
PRO low	0.0	mg/100g	Davies 1977	115.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**0**
PRO high	484.0	mg/100g	Davies 1977	115.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**968**
SER low	15.0	mg/100g	Davies 1977	105.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**30**
SER high	128.0	mg/100g	Davies 1977	105.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**256**

[1]0.2 is the approximate conversion from dry weight to fresh weight (internal communication)
[2]CLR=combined literature range

Free Amino Acids (Continued)

Substance	Value	Units	Reference	Formula Wt (FW)	Formula Used[1]	CLR[2] (ppm)
THR low	14.0	mg/100g	Davies 1977	119.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**28**
THR high	143.0	mg/100g	Davies 1977	119.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**286**
TRP low	7.0	mg/100g	Davies 1977	204.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**14**
TRP high	174.0	mg/100g	Davies 1977	204.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**348**
TYR low	9.0	mg/100g	Lisinska and Leszczynski 1989	181.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**18**
TYR high	319.0	mg/100g	Lisinska and Leszczynski 1989	181.2	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**638**
VAL low	15.0	mg/100g	Davies 1977	117.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**30**
VAL high	370.0	mg/100g	Davies 1977	117.1	mg/100g x 10mg/1000g x 1000g/kg x 0.2; mg/kg=ppm	**740**

[1]0.2 is the approximate conversion from dry weight to fresh weight (internal communication)
[2]CLR=combined literature range

Total Amino Acids						CLR[2]
Substance	Value	Units	Reference	Formula Wt (FW)	Formula Used[1]	Calc (ppm)
THR low	18.3	µM/g	Talley et al. 1984	119.1	µM/g x FW x 0.2	**436**
THR high	35.9	µM/g	Talley et al. 1984	119.1	µM/g x FW x 0.2	**855**
SER low	23.8	µM/g	Talley et al. 1984	105.1	µM/g x FW x 0.2	**500**
SER high	48.6	µM/g	Talley et al. 1984	105.1	µM/g x FW x 0.2	**1022**
PRO low	15.4	µM/g	Talley et al. 1984	115.1	µM/g x FW x 0.2	**355**
PRO high	63.6	µM/g	Talley et al. 1984	115.1	µM/g x FW x 0.2	**1464**
ALA low	22.0	µM/g	Talley et al. 1984	89.1	µM/g x FW x 0.2	**392**
ALA high	53.4	µM/g	Talley et al. 1984	89.1	µM/g x FW x 0.2	**952**
VAL low	32.1	µM/g	Talley et al. 1984	117.1	µM/g x FW x 0.2	**752**
VAL high	61.9	µM/g	Talley et al. 1984	117.1	µM/g x FW x 0.2	**1450**
ILE low	20.0	µM/g	Talley et al. 1984	131.2	µM/g x FW x 0.2	**525**
ILE high	36.3	µM/g	Talley et al. 1984	131.2	µM/g x FW x 0.2	**953**
LEU low	26.1	µM/g	Talley et al. 1984	131.2	µM/g x FW x 0.2	**685**
LEU high	52.7	µM/g	Talley et al. 1984	131.2	µM/g x FW x 0.2	**1383**
TYR low	12.6	µM/g	Talley et al. 1984	181.2	µM/g x FW x 0.2	**457**
TYR high	26.0	µM/g	Talley et al. 1984	181.2	µM/g x FW x 0.2	**942**
PHE low	16.7	µM/g	Talley et al. 1984	165.2	µM/g x FW x 0.2	**552**
PHE high	32.9	µM/g	Talley et al. 1984	165.2	µM/g x FW x 0.2	**1087**
HIS low	4.3	µM/g	Talley et al. 1984	155.2	µM/g x FW x 0.2	**133**
HIS high	15.1	µM/g	Talley et al. 1984	155.2	µM/g x FW x 0.2	**469**
ORN low	0.0	µM/g	Talley et al. 1984	136.16	µM/g x FW x 0.2	**0**
ORN high	1.7	µM/g	Talley et al. 1984	136.12	µM/g x FW x 0.2	**46**
LYS low	23.5	µM/g	Talley et al. 1984	146.2	µM/g x FW x 0.2	**687**
LYS high	46.8	µM/g	Talley et al. 1984	146.2	µM/g x FW x 0.2	**1368**
ARG low	20.1	µM/g	Talley et al. 1984	174.2	µM/g x FW x 0.2	**700**
ARG high	39.7	µM/g	Talley et al. 1984	174.2	µM/g x FW x 0.2	**1383**
TRP low	2.8	µM/g	Talley et al. 1984	204.2	µM/g x FW x 0.2	**114**
TRP high	6.9	µM/g	Talley et al. 1984	204.2	µM/g x FW x 0.2	**282**
ASP low	677	Mg/200g	Rogan et al. 2000	NA	mg/200g x 5 = mg/kg = ppm	**3385**
ASP high	1476	Mg/200g	Rogan et al. 2000	NA	mg/200g x 5 = mg/kg = ppm	**7380**
GLU low	583	Mg/200g	Rogan et al. 2000	NA	mg/200g x 5 = mg/kg = ppm	**2915**
GLU high	1207	Mg/200g	Rogan et al. 2000	NA	mg/200g x 5 = mg/kg = ppm	**6035**

[1]0.2 is the approximate conversion from dry weight to fresh weight (internal communication)
[2]CLR=combined literature range

Appendix 10

Post-Harvest Monitoring

INDEX

INTRODUCTION

All field trial sites are monitored for two growing seasons following the harvest in accordance with USDA regulations. The volunteer monitoring information for our 2009, 2010, and 2011 field trials are included in **Tables 1, 2, and 3** (up to date as of 21 June, 2012). During the monitoring period, the Trial Manager inspected the trial sites in accordance with the Simplot Design Protocol for volunteers. Any volunteers found in the trial sites were devitalized by using disking, roguing, spraying herbicide or as per design protocol.

RESULTS and DISCUSSION

A summary of all post-harvest monitoring pertaining to the 2009, 2010, and 2011 growing seasons is included in **Tables 1, 2, and 3**.

Table 1. 2010 and 2011 Post-Harvest Monitoring Data for 2009 Field Trials

Site Locations / USDA#	Monitoring Year 2010								
	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
Canyon County, ID/ 09-049-114n	NA	NA	0	0	0	0	0	0	0
Bingham County, ID/ 09-049-114n	NA	NA	0	0	0	0	0	0	0
Montcalm County, MI/ 08-353-104n	NA	NA	0	0	0	0	0	0	0
Cherry County, NE/ 09-077-112n	NA	NA	0	0	0	0	0	0	0
Oneida County, WI/ 09-049-114n	NA	NA	0	0	0	0	0	0	0
St Johns County, FL/ 08-353-103n	0	0	0	0	0	NA	NA	NA	NA
Site Locations	Monitoring Year 2011								
	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
Canyon County, ID/ 09-049-114n	NA	NA	0	0	0	0	0	0	0
Bingham County, ID/ 09-049-114n	NA	NA	0	0	0	0	0	0	0
Montcalm County, MI/ 08-353-104n	NA	NA	0	0	0	0	0	0	0
Cherry County, NE/ 09-077-112n	NA	NA	0	0	0	0	0	0	0
Oneida County, WI/ 09-049-114n	NA	NA	0	0	0	0	0	0	0
St Johns County, FL/ 08-353-103n	0	0	0	0	0	NA	NA	NA	NA

Table 2. 2011 and 2012 Post-Harvest Monitoring Data for 2010 Field Trials

Site Locations/ USDA#	Monitoring Year 2011								
	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
Bingham County, ID / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Canyon County, ID / 10-053-132n	NA	NA	0	0	+	+	+	0	0
St Johns County, FL 09-336-103n	0	0	0	0	0	0	0	0	0
Missaukee County, MI 10-053-132n	NA	NA	0	0	0	0	0	0	0
Montcalm County, MI 10-053-132n	NA	NA	0	0	0	0	0	0	0
Cherry County, NE / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Grand Forks County, ND / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Adams County, WA / 10-053-132n	NA	NA	0	+	+	+	0	0	0
Adams County, WI / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Oneida County, WI / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Vilas County, WI[1] 10-076-103n	NA	NA	0	0	0	0	0	0	0
Site Locations	Monitoring Year 2012								
	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
Bingham County, ID / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Canyon County, ID / 10-053-132n	NA	NA	0	15	6	1	2	0	0
St Johns County, FL / 09-336-103n	0	0	0	0	0	0	NA	NA	NA
Missaukee County, MI / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Montcalm County, MI / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Cherry County, NE / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Grand Forks County, ND / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Adams County, WA / 10-053-132n	NA	NA	0	0	4	+	0	0	0
Adams County, WI / 10-053-132n	NA	NA	0	0	0	0	0	0	0
Oneida County, WI / 10-053-132n	NA	NA	0	+	+	0	45	0	0
Vilas County, WI[1] / 10-076-103n	NA	NA	0	0	0	0	0	0	0

Table 3. 2012 Post-Harvest Monitoring Data for 2011 Field Trials

Site Locations/ USDA#	Monitoring Year 2012								
	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
Bingham County, ID / 11-063-103n	NA	NA	0	0	0	0	0	0	0
Canyon County, ID / 11-063-103n	NA	NA	0	15	18	27	7	0	0
St Johns County, FL / 10-326-103n	0	0	0	0	0	0	0	0	0
Pulaski County, IN/ 11-063-103n	NA	NA	0	20	TBD	0	0	0	0
Montcalm County, MI / 11-063-103n	NA	NA	0	0	0	0	0	0	0
Ionia County, MI / 11-150-101n	NA	NA	0	0	0	0	0	0	0
Cherry County, NE / 11-063-103n	NA	NA	0	0	0	0	0	0	0
Grand Forks County, ND/ 11-063-103n	NA	NA	0	0	0	0	0	0	0
Adams County, WA / 11-063-103n	NA	NA	0	0	0	0	0	0	0
Adams County, WI / 11-063-103n	NA	NA	0	0	0	0	0	0	0
Oneida County, WI/ 11-063-103n	NA	NA	0	0	1	0	0	0	0
Oneida County, WI/ 11-094-106n	NA	NA	0	0	0	0	0	0	0

+=Volunteers were found and destroyed

CONCLUSION

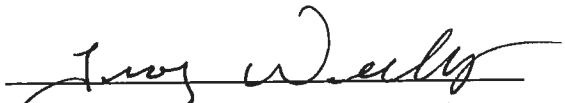
The data presented in **Tables 1**, **2**, and **3**, demonstrate that volunteer potato plants grown under the notifications were destroyed, in a similar fashion in fields that were planted and harvested the previous year.

It can be concluded that, based on post-harvest monitoring observations and other agronomic data presented elsewhere, the events do not pose a plant pest risk, and do not possess any characteristics that would lead to increased weediness.

CERTIFICATION

The undersigned certify that, to the best of their knowledge and belief, this appendix includes all data, information, and views relative to the matter, whether favorable or unfavorable to the position of the undersigned.

Sathya Adimulam, Quality Systems Manager, JR Simplot Company
5369 W Irving Street, Boise, ID 83706



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