Application to Food Standards Australia and New Zealand for the Inclusion of Potatoes with Late Blight Protection, Low Acrylamide Potential, Reduced Black Spot, and Lower Reducing Sugars in Standard 1.5.2 Food Produced Using Gene Technology

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SPS International Inc.

OECD Unique identifiers:

SPS-ØØØW8-4	**SPS-ØØE56-7**
SPS-ØØX17-5	**SPS-ØØF10-7**
SPS-ØØØY9-7	**SPS-ØØØJ3-4**

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This document does not contain confidential information

Part 1 General Requirements (3.1.1)

A. Executive Summary

SPS International Inc. (SPSII) has pioneered a new approach that uses Innate® technologies to transform potato plants with potato genomic DNA, without the incorporation of selectable markers or vector backbone sequences. SPSII has developed the Russet Burbank event SPS-∅∅∅W8-4, Ranger Russet event SPS-∅∅X17-5, and Atlantic event SPS-∅∅∅Y9-7, hereafter referred to as events W8, X17, and Y9, respectively. These events were developed to address the needs of the potato growers, industry and consumers for potatoes with late blight protection, lower acrylamide potential, reduced black spot, and lower reducing sugars.

The events W8, X17, and Y9 were developed by transforming the potato varieties Russet Burbank, Ranger Russet, and Atlantic, respectively, with pSIM1278 and pSIM1678. FSANZ has previously received a submission from SPSII for a potato event transformed with pSIM1278: Russet Burbank E12 (FSANZ Application Number A1128). FSANZ has not identified any public health and safety concerns in its assessment of the potato event E12.

The W8 event was developed by transforming Russet Burbank with pSIM1278 (Event E56) and subsequently transforming it with pSIM1678. W8 was developed independently from Russet Burbank E12, which is currently under assessment by FSANZ. Events similar to E12 (Ranger Russet F10, and Atlantic J3 and J55), containing a pSIM1278 insert, have previously been assessed and authorised by the U.S. and Canadian regulatory agencies, including USDA, FDA, Health Canada and Canadian Food Inspection Agency. The F10 and J3 events were transformed a second time with pSIM1678 to produce events X17 and Y9, respectively.

The T-DNA of pSIM1278 contains DNA sequences intended to down regulate endogenous enzymes through the mechanism of RNA interference (RNAi). The sequences were chosen from genes of enzymes present in potato tubers:

- *Asn1* (asparagine synthetase) for reduced free asparagine, contributing to low acrylamide potential
- *R1* (water dikinase) for lower reducing sugars, contributing to low acrylamide potential
- *PhL* (phosphorylase-L) for lower reducing sugars, contributing to low acrylamide potential
- *Ppo5* (polyphenol oxidase-5) for reduced black spot.

The pSIM1678 T-DNA contains the late blight resistance gene *Rpi-vnt1.* Late blight, caused by the oomycete *Phytophthora infestans* (*P. infestans*), is a serious disease of potatoes. The *Rpi-vnt1* gene produces the VNT1 resistance protein (R-protein), found in the wild *Solanum* species, *Solanum venturii* and *Solanum phureja*, which protects against foliar late blight. The VNT1 protein does not have a pesticidal mode of action, but rather enables the potato plant to detect a *P. infestans*-specific effector, Avr-Vnt1, and initiate its native immune response. In addition, the T-DNA of pSIM1678 contains potato vacuolar invertase (*VInv*) DNA sequence designed to down regulate the potato vacuolar invertase enzyme though RNAi, resulting in lower reducing sugars.

The W8, X17, and Y9 events with the desired modified traits were characterised and are the subject of this submission. In addition, SPSII asks that the *Australia New Zealand Food Standards Code* be amended to include events E56, F10 and J3, which are the primary events for W8, X17 and Y9, respectively.

The levels of free amino acids, reducing sugars, PPO activity, and field late blight protection were measured as an assessment of trait efficacy. These results demonstrated that W8, X17, and Y9 have

reduced levels of free asparagine and lower levels of reducing sugars compared to their controls. The changes to levels of free amino acids and reducing sugars are not nutritionally consequential as they do not affect the levels of essential amino acids or other key nutrients important to potato (OECD, 2002). The significantly lower levels of free asparagine and reducing sugars resulted in lower acrylamide in fries and chips made from W8, X17, and Y9 tubers. Additionally, the efficacy testing for PPO down regulation confirmed that PPO activity was significantly reduced in W8, X17, and Y9 tubers, consistent with effective down regulation of PPO in each event. Lastly, the late blight field efficacy studies confirmed that events W8, X17, and Y9 are each significantly more resistant to four strains of late blight than the Russet Burbank, Ranger Russet, and Atlantic control varieties.

Molecular characterisation of the events was performed to determine the number of copies, arrangement, and stability of the inserted DNA from both vectors. The events were confirmed to be free of *Agrobacterium*-derived backbone DNA. In the United States, confined field trials were undertaken, with the conventional variety and other cultivated varieties used as controls. Results from these trials confirmed no changes were observed that could have an impact on the environment or affect genetic stability. Compositional analysis was performed on field-grown tubers to compare nutritional and anti-nutritional compounds and showed no biologically relevant differences existed that could result in increased risk to humans or other non-target organisms. Analysis of the VNT1 protein and putative polypeptides produced from the inserted DNA indicated there are no sequences with significant homology to known allergens or toxins in these Innate® potatoes.

Analysis of the events W8, X17, and Y9 have not revealed any biologically relevant differences compared to the conventional varieties, except for the intended late blight protection, low free asparagine, low reducing sugars, and low polyphenol oxidase activity. Collectively, results of the molecular characterisation, agronomic assessment, and composition analysis support this application for amendment to the *Australia New Zealand Food Standards Code* to allow inclusion of the Innate® potato events W8, X17, and Y9 as well as their primary events E56, F10 and J3 respectively in **Standard 1.5.2**-*Food Produced Using Gene Technology*.

B. Applicant Details

(a)	Applicant's name/s	[redacted] (Regulatory Manager)
(b)	Company/organisation name	SPS International Inc.
(c)	Address (street and postal)	999 W Main St #1300, Boise, ID 83702, USA
(d)	Telephone number	+ [redacted]
(e)	Email address	[redacted]
(f)	Nature of the applicant's business	SPS International Inc. primarily files applications for approval of Simplot biotech events in international markets.
(g)	Details of other individuals, companies or organisations associated with the application	PTM Solutions Australia Pty Ltd 11 Moras Court Gisborne, VICTORIA 3437 Simplot@ptmsolutions.com.au

C. Purpose of the Application

This application seeks to amend the *Australia New Zealand Food Standards Code* to allow for the inclusion of potato events W8, X17, and Y9 in **Standard 1.5.2**-*Food Produced Using Gene Technology*.

SPSII has developed and tested potato events that have reduced expression of five enzymes and express a wild potato gene that conveys foliar protection against late blight. The potato events described in this application have the unique OECD codes: SPS-∅∅∅W8-4, SPS-∅∅X17-5 and SPS-∅∅∅Y9-7 and are referred to as W8, X17, and Y9 in this submission. SPSII currently does not intend to import the potatoes into Australia or New Zealand. The primary aim of this application is to obtain a food safety approval to protect international trade. This submission is consistent with SPS membership in the Excellence Through Stewardship® (ETS) program, adhering to stewardship and industry best practice by obtaining regulatory approvals in production and import markets.

In addition, SPSII asks that the *Australia New Zealand Food Standards Code* be amended to include E56, F10 and J3, which are the primary events for W8, X17 and Y9, respectively. The molecular characterisation of these primary events will be reviewed as part of the W8, X17 and Y9 safety reviews. Events E56, F10, and J3 contain only the quality traits of one insert and do not have the late blight protection or the lower vacuolar invertase traits of the second insert. To facilitate this decision, the unique OECD codes for these events are: SPS-∅∅E56-7, SPS-∅∅F1∅-7, SPS-∅∅∅J3-4. The insertions are stable, and composition data for X17 and Y9 have been included (Reports 15-61-SPS-COMP and 15-62-SPS-COMP). Event E56 does not have composition data and has not been commercialized to date. E56 is in confined field trials in the United States. The E56, F10, and J3 events all have limited commercial importance and the primary purpose for obtaining approval is to protect international trade.

D. Justification for the Application

SPS International Inc. has developed new potato events, W8, X17, and Y9. The new potato events were created using inserts containing potato DNA sequences that confer lower levels of free asparagine and reducing sugars, which together contribute to reduced acrylamide potential, and lower levels of polyphenol oxidase which reduces black spot. They also contain a wild potato gene that confers late blight protection.

Late Blight Protection: Late blight caused by the oomycete *Phytophthora infestans*, is a devastating disease among cultivated *Solanaceae* species. If left untreated, late blight affects potato foliage and tubers causing rapid necrosis and crop loss. The Irish potato famine was the result of late blight and illustrates the destructive nature of the disease. The cultivation of events W8, X17, and Y9 will enable growers to use an improved disease control strategy including integrated pest management (IPM) to manage late blight effectively.

Reduced Black Spot in Potatoes: Black spot refers to the black or greyish colour that may form in damaged or cut potatoes. It is a post-harvest physiological effect resulting from the handling of potato tubers during harvest, transport, processing, and storage and it contributes to waste experienced by growers, consumers, and processors. The enzymatic discoloration is associated with polyphenol oxidase (PPO) and occurs when the enzyme leaks out of the plastids of potatoes (Vaughn et al., 1988). Potatoes with black spot are either trimmed or rejected before processing, resulting in quality control challenges and economic loss. Lowering PPO levels in potatoes reduces the occurrence of black spot and this reduces grower, consumer, and processor waste.

Reduced Acrylamide Potential: Lowering the acrylamide potential of potatoes is important because acrylamide presents a potential health risk for consumers (FDA, 2013). Although acrylamide is not present

in fresh potatoes, it forms in carbohydrate-rich foods when the amino acid asparagine and the reducing sugars, glucose and fructose, are heated at temperatures above 120 °C (O'Brien and Morrissey, 1989). Lowering the concentrations of free asparagine, glucose, and fructose in potatoes reduces the acrylamide potential of cooked potatoes. The biochemical basis of acrylamide formation has been published by Stadler, 2005.

Potatoes with lower acrylamide address a potential health concern for consumers, especially in light of recent toxicology studies (Food Drink Europe[1], 2014; Health Canada, 2012; NTP, 2012). Various governments have responded to the findings of acrylamide in food by providing guidance documents and conducting surveys to assess the source and intake of acrylamide in foods (EFSA CONTAM Panel, 2015; FDA, 2006; Food Drink Europe, 2014).

The United States FDA has proposed guidance for industry on the reduction of acrylamide levels in food products (FDA, 2013). In their *Draft Guidance for Industry on Acrylamide in Foods*, the FDA notes "Reducing acrylamide in foods may mitigate potential human health risks from exposure to acrylamide." An extensive list of potential mitigation techniques were summarised in the guidance document (FDA, 2013), focusing primarily on the reducing sugar levels in potatoes. Many of the methods in FDA's Guidance document are consistent with those reported in the *Acrylamide Toolbox* published by Food Drink Europe (Food Drink Europe, 2014).

Estimated dietary exposures of Australian consumers to acrylamide in food were investigated as a part of the first phase of the 24th Australian Total Diet Study (FSANZ, 2014). The study found that the levels of acrylamide were generally lower than, or comparable to, those reported in previous Australian and international studies. However, the estimated dietary exposures of Australian consumers were in the range considered to be of possible concern to human health by the Joint Expert Committee on Food Additives.

In New Zealand, the Ministry for Primary Industries (MPI) reassessed dietary exposure with a survey of foods contributing to acrylamide intake in New Zealand (Cressey et al., 2012). The survey found that dietary exposure estimates have remained fairly constant since a previous survey in 2006.

International food regulators are working with industry to reduce acrylamide levels. New farming and processing techniques are being investigated to produce lower levels of acrylamide, for example, lowering cooking temperatures, using enzymes that reduce acrylamide formation, and obtaining raw materials with lower reducing sugar levels.

FSANZ is encouraging and supporting industry to use enzymes that reduce acrylamide formation and urging industry to adopt an *Acrylamide Toolbox* produced by the Confederation of the Food and Drink Industries of the EU (CIAA, 2013). A Codex working group has created a Code of Practice for reducing acrylamide in food (Codex, 2009). Both FSANZ and MPI contributed to the development of this Code of Practice. The W8, X17, and Y9 events satisfy these recommendations by providing a product with lower levels of reducing sugars and free asparagine. These characteristics result in significantly lower levels of acrylamide in processed potato products.

E. Information to Support the Application

This application consists of 2 parts containing information in accordance with the following checklists:

- Part 1: General requirements (3.1.1)

[1] Food Drink Europe, 2014 (http://www.fooddrinkeurope.eu/publications/category/toolkits/)

- Part 2: Foods produced using gene technology (3.5.1) main document, Part 2 information. Supplement form molecular analysis.

F. Assessment Procedure

SPSII is anticipating that this application will be considered under the **General Procedure** for Administrative Assessment process by Food Standards Australia New Zealand.

G. Confidential Commercial Information (CCI)

Confidential Commercial Information (CCI) has not been included in this submission document.

Release of Information

SPSII is submitting the information in this application for review by Food Standards Australia New Zealand (FSANZ) for amendment to the Food **Standard 1.5.2** Food Produced Using Gene Technology. SPSII holds proprietary rights to the extent allowable by law to all such information and by submitting this information, SPSII does not authorise its release to any third party except to the extent it is duly requested under the Freedom of Information Act 1982 (*FOI Act*) or in compliance with the responsibility of FSANZ to publish documents required under Sections 8, 8(A), 8(C) and 8(D) of the *FOI Act*; and this information is responsive to the specific aforementioned request. Accordingly, except as specifically stated above, SPSII, does not authorise the release, publication or other distribution of this information (including website posting or otherwise), nor does SPSII authorise any third party to use, obtain, or rely upon this information, directly or indirectly, as part of any other application or for any other use, without SPSII's prior notice and written consent. Submission of this information does not in any way waive SPSII's rights (including rights to exclusivity and compensation) to such information.

H. Other Confidential Information

No additional confidential material is included in this submission document.

I. Exclusive Capturable Commercial Benefit

SPSII acknowledges that the proposed amendment to the Standard will likely result in an exclusive capturable commercial benefit being accrued to the parent company (J.R. Simplot Company) as defined in Section 8 of the *FSANZ Act*.

Costs and Benefits

Today, one of the main global challenges is how to ensure food security for a growing population whilst ensuring long-term sustainable development. According to the FAO, food production will need to grow by 70% to feed world population, predicted to reach 9 billion by 2050 (Alexandratos and Bruinsma, 2012). Current trends, such as increasing urban population, shift of lifestyle and diet patterns of the rising middle class in emerging economies, along with climate change, put considerable pressure on the earth's resources.

In the meantime, while food insecurity remains unacceptably high, each year, massive quantities of food are lost worldwide due to spoilage and infestations on the journey from farm to consumers. One of the major ways of strengthening food security is by reducing these post-harvest losses.

The term 'post-harvest loss' refers to measurable quantitative and qualitative food loss in the post-harvest supply chain. The supply chain comprises interconnected activities from the time of harvest through crop processing, marketing and food preparation, to the final decision by the consumer to eat or discard the food.

Post-harvest loss reduction interventions are a critical component of efforts to reduce food insecurity, as part of an integrated approach to realising agriculture's full potential to meet the world's increasing food and energy needs. Therefore, reducing post-harvest loss by making more effective use of today's crops, improving productivity on existing farmland, and bringing additional acreage into sustainable production, is critical to facing the challenge of feeding an increasing world population.

As a global staple food crop, post-harvest losses in the potato supply chain due to black spot, enzymatic darkening, cause waste and economic loss. These issues occur in the fresh and processed food supply chains in both industrial and third world countries across the globe.

Enzymatic darkening is a widespread colour reaction occurring in fruits and vegetables, which involves the interaction of oxygen, phenolic compounds and polyphenol oxidases (PPOs). Darkening is usually initiated by bruising of the potato caused by impact and pressure during harvest and storage. It also is initiated by slicing/dicing/juicing fresh fruit and/or vegetables for use in fresh consumption or as part of preparation for further processing. As a result, PPO catalyses the enzymatic oxidation and conversion of monophenols to o-diphenols and o-dihydroxyphenols to o-quinones. The quinone products polymerise and react with amino acid groups of cellular proteins, resulting in black or brown pigment deposits ('darkening').

A variety of fruits and vegetables, such as apple, pear, banana, peach, lettuce and potato, are especially susceptible to enzymatic darkening during storage and processing. Darkening has a negative effect on appearance and may impair other sensory properties including taste, odour and texture.

Significant savings can be realised both economically and environmentally, because events W8, X17, and Y9 have reduced black spot. For example, if W8 replaced all conventional Russet Burbank potatoes in the United States fresh market, potato waste would be reduced by 635 million kg annually, resulting in savings of $90 million in producer costs. This amount of waste reduction would translate to an annual reduction of 27 million kg of CO_2 emissions, of 25 billion fewer litres of water and of up to 69,000 hectares of pesticide applications (Halterman et al., 2016). These estimates are based on the reduced acres needed to achieve the same marketable yield that would result from W8 replacing conventional Russet Burbank potatoes in the fresh market.

The W8, X17, and Y9 potatoes use RNAi gene silencing technology to regulate the expression of the genes responsible for the enzymatic darkening process. As a result, Innate® potatoes are less susceptible than conventional potatoes to darkening and the onset of black spot from bruising caused by impact and pressure during harvest, storage and food preparation.

Research has demonstrated potential for the following benefits to be captured by United States potato farmers, supply chain participants and consumers following the introduction of Innate® technology in a range of potato varieties.

- Innate® potatoes reduce bruise and black spot up to 44% compared to conventional varieties.
- Using Innate® technology, it is possible for packers to experience an estimated 15% increased pack-out of fresh-grade potatoes, providing better utilisation, improved processing efficiencies and less waste.
- Because Innate® potatoes show less bruise, there will be fewer rejected loads by processors and a reduction in price discounts based on quality downgrades due to bruising.

- Consumers will throw away fewer fresh potatoes – it is estimated that up to 35% of fresh potatoes are wasted in the United States alone, representing 1.67 billion kg per year which has been estimated to cost upwards of $1.7 billion annually (Buzby et al., 2011) United States Journal of Consumer Affairs.
- Food approvals in influential countries like Australia increase the likelihood that these potatoes can become available in developing countries to help alleviate hunger in resource-scarce parts of the world. Simplot is working to help ensure this can happen by partnering with Michigan State University to develop late blight protected varieties for Bangladesh and Indonesian farmers. This initiative is part of the United States Government's Feed the Future initiative.

In addition to reduced black spot, the W8, X17, and Y9 potatoes have lower levels of free asparagine and reducing sugars, which decreases the potential for acrylamide formation. Acrylamide is a chemical compound that occurs when potatoes, wheat, coffee, and other foods are cooked at high temperatures. The United States FDA and the European Food Safety Authority have classified acrylamide as a probable carcinogen. Many international and national regulatory agencies advise limiting dietary intake of acrylamide.

Most potatoes consumed in Australia are grown domestically. Domestic production of potato in Australia (2013/14 - 1,171,300 metric tonnes) is supplemented by imports of processed potato, predominantly from the United States (2012/13 – 108,623 metric tonnes). Australian potato exports were 52,371 tonnes for the year ending June 2013. This was made up of 37,766 tonnes fresh product and 14,605 tonnes processed product.

Of the total domestic production, 53% is sent to processing while the remaining 47% is sold fresh. When local processed volumes are combined with the 108,622 tonnes of imported product, a total of 412,343 tonnes is supplied to the processing sector. The majority (79%) of this is distributed to foodservice outlets, while 19% is distributed to retail outlets and 4% is exported.

Potatoes enjoy consistent market penetration year-round at 52-58%, confirming their role as a staple Australian product on both summer and winter menus. Family households typically buy more potatoes at each purchase than smaller households, while households with lower discretionary income are more likely to purchase lower priced pre-packed products compared to those with higher discretionary income. (AusVeg[2], August 2015)

In Australia, consumer research has confirmed that all major uses of potato involve some level of cooked preparation. The versatility of potato has led to a range of uses, with the majority being centred around dinner, and to lesser degree lunch time occasions. Seasonal influences have little bearing on the main uses of potato, reflecting patterns of consistent consumption year round. Within the Australian diet, potatoes are prepared and consumed in the following forms:

- Boiled, Microwaved, Steamed;
- Deep fried;
- Mashed;
- Roasted;
- Baked/Grilled;
- Salad – cooked;
- Soup/Sauce;

[2] Ausveg's Potato Consumer Research http://ausveg.com.au/potatoes/potato-consumer-research.htm.

- Stir fry;
- Juiced;
- Sandwich/burger/wrap; and
- Snacks – potato chips / straws.

In Australia, for the year ending June 2013, per capita consumption for fresh potatoes purchased through retail for home consumption was 14.56 kg, and for fresh potatoes purchased and consumed in food service away from home was 3.24 kg. Per capita consumption for processed potato products purchased through retail for home and foodservice consumption was 17.73 kg. Therefore, total annual consumption was 35.53 kg per capita (Fresh Logic, 2014). In New Zealand, average consumption was estimated at 22.89Kg per capita (Potatoes NZ, 2014).

The importance of potato and the impact of black spot darkening in the fresh potato market is demonstrated in Ausveg's Potato Consumer Research of August 2015 that reported that - "Consumers are concerned about wastage and are seeking information on freshness (best before dates). This could be in the form of providing estimated freshness for loose potatoes at the point of sale, such as 'will last for 2 weeks in your cupboard."

The events W8, X17, and Y9 also contain the *Rpi-vnt1* gene from *S. venturii* that confers foliar protection to an important potato pathogen, *P. infestans*, the causal agent of late blight (Foster et al., 2009). Late blight is a devastating disease among cultivated *Solanaceae* species. In potato, late blight affects foliage and tubers causing rapid necrosis and crop loss if left untreated (Haverkort et al., 2008). The Irish potato famine was the result of late blight and demonstrates the destructiveness of the disease (FAO, 2008). Symptoms appear at first as water-soaked spots on leaves. Inside the infected leaves, sporangiophores emerge through the stomata of the stems and leaves and produce sporangia. The sporangia when ripe become detached, and are easily spread by wind and rain causing new infections (Agrios, 2004). During irrigation or wet weather conditions, sporangia are washed down from the leaves and into the soil where they subsequently penetrate and infect the tubers (Agrios, 2004). Infected tubers will likely rot in storage, contaminate equipment, and spread infection to non-infected tubers (Miller et al., 2006). Under favorable conditions for late blight (i.e. humidity, temperature, and host susceptibility), asexual reproduction of the pathogen occurs in as few as four days leading to disease epidemics (Agrios, 2004).

Nine of the top ten registered seed varieties grown in Canada are susceptible to late blight, comprising approximately 66% of planted acreage (CFIA, 2015; NPC, 2015). Therefore, IPM practices to control late blight rely primarily on chemical fungicides, crop rotation, and other cultural controls to minimize risk of infection (Canadian Horticulture Council, 2011; Miller et al., 2006). The Pest Management Centre of Agriculture and Agri-Food Canada recommend both a prophylactic spray fungicide program and field rotations with crops such as cereals and forages in order to minimize the occurrence of disease and improve soil and crop productivity (Agriculture and Agri-Food Canada, 2005). The planting of late blight resistant varieties allows the use of a balanced disease control strategy that employs IPM programs while maintaining durability of the trait.

Resistance genes (R-genes) in wild *Solanum* species provide natural protection to late blight (Sedlák et al., 2005). These genes can be bred or introduced into new varieties that then become resistant to late blight. Potato breeding programs from the mid-twentieth century used *Solanum demissum*, a wild potato species found in central Mexico and a good source of protection against certain strains of late blight (Pel, 2010; Vleeshouwers et al., 2011). *S. demissum* R-genes designated *R1-R11* have been identified, some of which have been widely used for introgression in European breeding programs to help control late blight (Malcolmson and Black, 1966).

Numerous research studies have been carried out worldwide to develop late blight resistant potato cultivars and improve their durability. The introduction of new cultivars containing these R-genes was initially successful, but rapidly evolving populations of *P. infestans* reduced their efficacy (Fry et al., 2015). Durability in the field of a particular R-gene is variable (Leach et al., 2001), and additional novel resistance genes against *P. infestans* are being discovered from other wild *Solanum* species. Genes from wild *Solanum* species have been integrated into many edible potato cultivars through breeding with wild species such as *S. bulbocastanum* (Park et al., 2005), S*. stoloniferum* (Haverkort et al., 2008; Hutten et al., 2013), *S. microdontum* (Tan et al., 2008) and *S. phureja* (Śliwka et al., 2013).

Potatoes are highly heterozygous and subject to inbreeding depression, so many years are required to develop new varieties through conventional breeding. Due to these challenges, potato varieties do not have a high frequency of introduction and discontinuation. Because it is difficult to breed and backcross traits into potatoes, biotech offers a mechanism to modify traits while maintaining desired traits in a variety.

The potato industry is a substantial and important industry across both Australia and New Zealand. Potatoes are the highest value horticultural crop grown for consumption in Australia and are grown in all states of Australia except the Northern Territory. In New Zealand, potatoes are grown in Pukekohe, Waikato, Hawkes Bay, Manawatu, Canterbury and Southland.

The Australian National Potato Industry Biosecurity Plan (the Potato Biosecurity Plan) was developed by Plant Health Australia (PHA) in collaboration with industry and government stakeholders and was launched in May 2007 and updated in 2013. The Potato Biosecurity Plan notes that Australia's geographic isolation and lack of shared land borders have, in the past, provided a degree of natural protection from exotic threats.

The Potato Biosecurity Plan lists Late blight – A2 mating type – *Phytophthora infestans*, as one of the top-ranked pest threats to the Australian potato industry. The events W8, X17, and Y9 currently are not intended for the Australian market, however, such technology represents future opportunities for Australian growers and consumers.

J. International and Other National Standards

Applications for approval of SPS-∅∅∅W8-4, SPS-∅∅X17-5 and SPS-∅∅∅Y9-7 have been submitted to other jurisdictions (

Table 1).

Responsible environmental stewardship and deployment of biotechnology-derived products are important to SPS International Inc., to its parent company the J.R. Simplot Company, and to Simplot Plant Sciences (SPS), the biotechnology group within the J.R. Simplot Company. SPS is a member of Excellence Through Stewardship® (ETS), an industry-coordinated initiative that promotes the global adoption of stewardship programs and quality management systems for the full life cycle of biotechnology-derived plant products. The ETS "*Guide for Product Launch Stewardship of Biotechnology-Derived Products*" (ETS, 2013) also references and is consistent with the product launch policies of the Biotechnology Industry Organisation and Crop Life International.

Table 1: Current Applications and Approval Status for SPS-ØØE56-7, SPS-ØØF10-7 and SPS-ØØØJ3-4, SPS-ØØØW8-4, SPS-ØØX17-5 and SPS-ØØØY9-7

Country	Competent National Authority	Type of Authorisation	Approval Status					
			E56-7	F10-7	J3-4	W8-4	X17-5	Y9-7
United States	United States Department of Agriculture (USDA)	Determination of non-regulated status	-[a]	Approved	Approved	Approved	Under Review	
	Food and Drug Administration (FDA)	Food and feed safety assessment	-	Approved	Approved	Approved	Under Review	
	Environmental Protection Agency (EPA)	Permanent exemption from Tolerance to VNT1; Event Registration	Not applicable	Not applicable	Not applicable	Under Review		
Canada	Canadian Food Inspection Agency (CFIA)	Unconfined environmental release	-	Approved	Approved	Under Review		
		Use in livestock feed	-	Approved	Approved	Under Review		
	Health Canada	Food approval	-	Approved	Approved	Under Review		

[a] Event E56 has not been commercialised

The Codex *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* (CAC/GL 45-2003, 2006) is applicable to the assessment of this application to amend the *Australia New Zealand Food Standards Code* to allow for the inclusion of events SPS-ØØØW8-4, SPS-ØØX17-5, SPS-ØØØY9-7 and their primary events SPS-ØØE56-7, SPS-ØØF10-7 and SPS-ØØØJ3-4 respectively in **Standard 1.5.2**–Food Produced Using Gene Technology.

K. Statutory Declaration – Australia

Statutory Declarations Act 1959

I, [redacted] for SPS International Inc., 999 W Main St #1300, Boise, ID 83702, United States of America, make the following declaration under the *Statutory Declarations Act 1959*:

1. the information provided in this application fully sets out the matters required
2. the information provided in this application is true to the best of my knowledge and belief
3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

[redacted]

Declared at Simplot Plant Sciences on 9th of November, 2016
[place] [day] [month] [year]

Before me, [redacted]

[redacted]

Signature of person before whom this declaration is made

..... [redacted] .....

[Full name, qualification and address of person before whom this declaration is made]

L. Checklists Provided With Application

General Requirements

General requirements (3.1.1)		
Check	**Page No.**	**Mandatory requirements**
☒	2	A Form of application ☒ Application in English ☒ Executive Summary (separated from main application electronically) ☒ Relevant sections of Part 3 clearly identified ☒ Pages sequentially numbered ☒ Electronic copy (searchable) ☒ All references provided
☒	3	B Applicant details
☒	4	C Purpose of the application
☒	4	D Justification for the application ☒ Regulatory impact information ☒ Impact on international trade
☒	5	E Information to support the application ☒ Data requirements
☒	6	F Assessment procedure ☒ General ☐ Major ☐ Minor ☐ High level health claim variation
☒	6	G Confidential commercial information ☐ CCI material separated from other application material ☐ Formal request including reasons ☐ Non-confidential summary provided
☒	6	H Other confidential information ☐ Confidential material separated from other application material ☐ Formal request including reasons
☒	6	I Exclusive Capturable Commercial Benefit ☒ Justification provided
☒	10	J International and other national standards ☒ International standards ☒ Other national standards
☒	12	K Statutory Declaration
☒	13	L Checklist/s provided with application ☒ 3.1.1 Checklist ☒ All page number references from application included ☒ Any other relevant checklists for Chapters 3.2–3.7

Foods Produces Using Gene Technology

Foods produced using gene technology (3.5.1)		
Check	**Page No.**	**Mandatory requirements**
☒	26	A.1 Nature and identity
☒	27	A.2 History of use of host and donor organisms
☒	33	A.3 Nature of genetic modification
☒	83	B.1 Characterisation and safety assessment
☒	115	B.2 New proteins
☒	115	B.3 Other (non-protein) new substances
☐	N/A	B.4 Novel herbicide metabolites in GM herbicide-tolerant plants
☒	126	B.5 Compositional analyses
☒	143	C Nutritional impact of GM food
☒	143	D Other information

Part 2 Specific Data Requirements for Safety Assessment

The following information is provided to support an application for a new genetically modified food. The details presented are in accordance with Section 3.5.1. of the FSANZ Application Handbook as at 1 March 2016.

Table of Contents

List of Tables

List of Figures

Abbreviations, Acronyms and Definitions[3]

Abbreviation	Definition
ADP	Adenosine diphosphate
AGP	Probe used to detect *Agp* promoter sequence
Agp	ADP-glucose pyrophosphorylase gene
A. tumefaciens	*Agrobacterium tumefaciens*
AP1	Adapter primer 1
AP2	Adapter primer 2
ASN	Probe used to detect *Asn1* sequence
ASN1	*Asn1* gene-derived probe used in DNA gel blot hybridization
Asn1	Asparagine synthetase-1 gene
ATP	Adenosine triphosphate
AUDPC	Area under disease progress curve
AUG	Start codon
Backbone DNA	DNA associated with construct backbone
BB1-BB8	Probes that detect pSIM1278/pSIM1678 backbone regions
bp	Base pair
CC	Coiled-coil domain
CFR	Code of Federal Regulations
CPC	Canadian Potato Council
cwt/A	Unit of measure equal to 100 lbs/acre or weight (lbs) of tubers harvested/acre divided by 100
DEEM	Dietary exposure evaluation model
DIGII	Molecular weight markers (125 bp – 23,130 bp)
DIGVII	Molecular weight markers (81 bp – 8,576 bp)
DNA insert	DNA sequence from pSIM1278 or pSIM1678 integrated into the potato genome
dNTP	Deoxy nucleotide triphosphate
dsRNA	Double-stranded RNA
ETS	Excellence Through Stewardship
FARRP	Food Allergy Resource Research Program and the University of Nebraska Lincoln
FDA	Food and Drug Administration
GBS, GBS1	Probe used to detect one region of the *Gbs* promoter sequence
GBS2	Probe used to detect second region of the *Gbs* promoter sequence
Gbss	Granule-bound starch synthase gene
IB	Internal band
ILSI	International Life Sciences Institute
Innate®	A branded biotechnology approach that uses plant genes to enhance desired traits
INV	Probe used to detect *VInv* sequence
IPM	Integrated pest management
ipt	Isopentenyltransferase gene—produces cytokinin hormones associated with plant growth and development

[3] NOTE: Abbreviations of units of measure and of physical and chemical quantities are used according to the standard format described in Instructions to Authors in the Journal of Biological Chemistry (http://www.jbc.org/).

JB	Junction band
Kan^R	Kanamycin resistance gene provides a selectable marker for maintenance in bacteria
kb	Kilobase
LB	Left border
LOD	Limit of detection
LOQ	Limit of quantification
LRR	Leucine-rich repeat domain
MOPS	3-(*N*-morpholino)propanesulfonic acid
MSU	Michigan State University
N. benthamiana	*Nicotiana benthamiana*
NB	Nucleotide-binding
NB-VNT1	full-length VNT1 expressed and enriched from *N. benthamiana*
NBY	Nutrient broth-yeast extract
NBS-LRR	Nucleotide binding site/ leucine rich repeat
NGS	Next generation sequencing
NTO	Non-target organism
OECD	Organisation for Economic Cooperation and Development
ORF	Open reading frame
P. infestans	*Phytophthora infestans*
pAgp	Promoter of the ADP glucose pyrophosphorylase gene
pBR322	Backbone element
PCR	Polymerase chain reaction
pGbss	Promoter of the granule-bound starch synthase gene
pGEM-T	Cloning vector
PhL	Phosphorylase-L gene
PPO	Polyphenol oxidase enzyme
Ppo5	Polyphenol oxidase-5 gene
qPCR	Quantitative PCR
pVnt1	*Rpi-vnt1* promoter
pVS1	Backbone element
R-protein	Resistance protein
R-gene	Resistance gene
R1	Southern blot probe used to detect the R1 cassette
R1	Water dikinase R1 gene
RB	Right border
Rpi-vnt1	Protection gene against *P. infestans*
RT-qPCR	Reverse transcription-qualitative polymerase chain reaction
S. phureja	*Solanum phureja*
S. tuberosum	*Solanum tuberosum*
S. venturii	*Solanum venturii*
SDS	Sodium dodecyl sulfate
Somaclonal variation	Genetic and/or phenotypic variation among propagated plants from a single parent arising from the callus phase of tissue culture
SPS-∅∅E56-7	OECD identifier for E56
SPS-∅∅F10-7	OECD identifier for F10
SPS-∅∅∅J3-4	OECD identifier for J3
SPS-∅∅∅W8-4	OECD identifier for W8

SPS-∅∅X17-5	OECD identifier for X17
SPS-∅∅∅Y9-7	OECD identifier for Y9
SSC	Saline sodium citrate
tVnt1	*Rpi-vnt1* terminator
Ubi7	Polyubiquitin 7 promoter
Ubi3	Polyubiquitin 3 terminator
USDA-APHIS	United States Department of Agriculture-Animal and Plant Health Inspection
VInv	Vacuolar invertase
VNT	Probe used to detect *Rpi-vnt1* sequence
VNT1	VNT1 protein
WT	Wild-type

A. Technical Information on the Food Produced Using Gene Technology

A.1. Nature and Identity of the Genetically Modified Food

A.1(a) A description of the GM organism from which the new GM food is derived. The description must include the nature and purpose of the genetic modification.

The events W8, X17, and Y9 were developed by transforming the potato varieties Russet Burbank, Ranger Russet, and Atlantic, respectively, with the *Agrobacterium* vectors pSIM1278 and pSIM1678. The events were developed to confer late blight protection, lower levels of free asparagine and reducing sugars, and reduced polyphenol oxidase, which contribute to reduced acrylamide potential and reduced black spot (Table 2).

The vector pSIM1278 contains two cassettes for the down-regulated expression of four specific potato enzymes (asparagine synthetase, polyphenol oxidase, water dikinase, and phosphorylase). The vector pSIM1678 also contains two cassettes, one for the expression of a wild potato gene to convey late blight protection and one for the down regulated expression of a specific potato enzyme vacuolar invertase.

Table 2: Summary of Genes, Intended Traits, and Benefits in W8, X17, and Y9

Construct	Gene Target	Mechanism	Intended Trait	Intended Benefit
pSIM1278	*Asn1*: asparagine synthetase-1[1]	RNAi down regulation	Reduces free asparagine	Contributes to low acrylamide potential[2]
	R1: water dikinase[1]	RNAi down regulation	Lowers reducing sugars	Contributes to low acrylamide potential[2]
	PhL: phosphorylase-L[1]	RNAi down regulation	Lowers reducing sugars	Contributes to low acrylamide potential[2]
	Ppo5: polyphenol oxidase-5[1]	RNAi down regulation	Reduces enzymatic darkening	Reduced black spot
pSIM1678	*VInv*: vacuolar invertase	RNAi down regulation	Lowers reducing sugars	Contributes to low acrylamide potential[2]
	Rpi-vnt1: R-gene	Protein expression	Confers protection against *P. infestans*	Late blight protection

[1] Currently being evaluated by FSANZ in Application A1128.
[2] Acrylamide is formed primarily from asparagine and reducing sugars heated at temperatures above 120 °C, as occurs during frying.

A.1(b) The name, number or other identifier of each of the new lines or strains of GM organism from which the food is derived.

In accordance with OECD '*Guidance for the Designation of a Unique Identifier for Transgenic Plants*', the OECD Unique Identification Code for the potato events are Russet Burbank event SPS-∅∅∅W8-4, Ranger Russet event SPS-∅∅X17-5, and Atlantic event SPS-∅∅∅Y9-7.

A.1(c) The name the food will be marketed under (if known).

The potatoes containing the Innate® technology will be marketed on the Canadian and United States fresh potato market as:

- Event W8–Glaciate;
- Event X17–Acclimate; and
- Event Y9–Hibernate.

These potatoes will be marketed under a variety of labels as fresh cut, fries or crisps, depending on the licenced user of the event.

A.2. History of use of the host and donor organisms

A.2(a) For the donor organism(s) from which the genetic elements are derived:

A.2(a)(i) Any known pathogenicity, toxicity or allergenicity of relevance to the food

Donor DNA in the insert consists of both coding and non-coding genetic elements from two plasmids pSIM1278 and pSIM1678 as described in Table 3 and Table 4. All sequences from pSIM1278 are from *Solanum tuberosum* var. Ranger Russet (Potato) except for 2 elements derived from *S. verrucosum,* a diploid (2n=24) wild potato species from Mexico. Similarly, all sequences from pSIM1678 are from *Solanum tuberosum* var. Ranger Russet (Potato) with the addition of the native *Rpi-vnt1* promoter, gene and termination sequence first cloned from a South American wild *Solanum* species, *S. venturii* (Foster et al., 2009; Pel, 2010).

Details of the pathogenicity, toxicity or allergenicity of potato are described in the OECD Consensus Document on Compositional Considerations for New Varieties of Potatoes: Key Food and Feed Nutrients, Anti-nutrients and Toxicants (OECD, 2002).

Potatoes are not known to cause disease in humans or animals and have a long history of safe use as a food. Several features of this commodity relate to toxicity and allergenicity, and are briefly discussed below.

Bioinformatic analyses confirm that VNT1 lacks significant homology to known toxins and allergens. Additionally, bioinformatic analyses demonstrate that proteins highly similar to VNT1 are present in the human diet, supporting a history of safe use. R-proteins like VNT1 do not confer pest protection by directly targeting the pest or by acting as toxins. Instead, VNT1 activates the plant's native, immune pathway resulting in a hypersensitive response. These factors contribute to the low hazard potential of VNT1.

All potatoes contain natural toxins called glycoalkaloids, the most prevalent of which are solanine and chaconine. Solanine is also found in other plants in the family Solanaceae, which includes plants such as as the edible crops eggplant and tomato.

Potatoes are not among the “Big Eight” group of foods that account for ~90% of all food allergies in the U.S. (FARRP, 2014). There are a few reports of allergies to cooked potato in children (De Swert et al., 2002, 2007). Patatin (Sol t 1) has been identified as the primary allergen involved in this reaction (Astwood et al., 2000). Patatin is a storage glycoprotein that displays lipase activity and makes up about 40% of the soluble protein in tubers (Mignery et al., 1988). Because potato protein naturally contains a relatively large proportion of patatin, any unexpected change in patatin levels would be unlikely to affect allergenicity enough to alter consumption patterns for people allergic to potatoes. Additionally, there is no mechanistic reason to suggest that the level of patatin would be changed in W8, X17, and Y9.

No sequences associated with either glycoalkaloids or patatin proteins were used in creating the potato events in this application.

ORDER:	Solanales
FAMILY:	Solanaceae
GENUS:	*Solanum*
SPECIES:	*S. tuberosum*, *S. verrucosum* Schltdl and *S. venturii*
COMMON NAME:	Potato, Wild Potato

Table 3. Genetic Elements of pSIM1278, from Left Border to Right Border

Genetic Element	*Origin*	Accession Number	Position (pSIM1278)	Size (bp)	Intended Function
1. Left Border (LB) site[1]	Synthetic	AY566555[2]	1–25	25	Secondary cleavage site releases ssDNA insert from pSIM1278 (van Haaren et al., 1989)
2. LB region sequence	*S. tuberosum* var. Ranger Russet	AY566555[2]	26–187	162	Buffer for truncations during insertion
3. Intervening Sequence	*S. tuberosum*	AF393847	188 –193	6	Sequence used for DNA cloning
4. ADP glucose pyrophosphorylase gene promoter (*pAgp*), 1st copy	*S. tuberosum* var. Ranger Russet	HM363752	194-2,453	2260	Drives expression of *Asn1* and *Ppo5* inverted repeats , especially in tubers
5. *Asn1* gene fragment (1st copy, antisense orientation)	*S. tuberosum* var. Ranger Russet	HM363759	2,454-2,858	405	Generates dsRNA that down regulates asparagine synthetase to impair asparagine formation (Chawla et al., 2012[3])
6. Fragment of the 3'-untranslated region of the polyphenol oxidase-5 gene (*Ppo5*) (1st copy, antisense orientation)	*S. verrucosum*	HM363754	2,859-3,002	144	Generates dsRNA that triggers the down regulation of PPO to reduce black spot development
7. Intervening Sequence	*S. tuberosum*	DQ478950	3,003-3,008	6	Sequence used for DNA cloning
8. Spacer-1	*S. tuberosum* var. Ranger Russet	HM363753	3,009-3,165	157	Sequence between the 1st inverted repeat; transcript forms loop in dsRNA
9. Fragment of the 3'-untranslated region of the polyphenol oxidase-5 gene (*Ppo5*) (2nd copy, sense orientation)	*S. verrucosum*	HM363754	3,166-3,309	144	Generates dsRNA that triggers the down regulation of PPO to reduce black spot development
10. *Asn1* gene fragment (2nd copy, sense orientation)	*S. tuberosum* var. Ranger Russet	HM363759	3,310-3,715	406	Generates dsRNA that down regulates asparagine synthetase to impair asparagine formation (Chawla et al., 2012[3])
11. Intervening Sequence	*S. tuberosum*	X73477	3,716-3,721	6	Sequence used for DNA cloning
12. Granule-bound starch synthase gene promoter (*pGbss*) (opposite direction from 1st copy of *pAgp*)	*S. tuberosum* var. Ranger Russet	HM363755	3,722-4,407	686	Drives expression of *Asn1* and *Ppo5* inverted repeats, especially in tubers
13. Intervening Sequence	*S. tuberosum*	X95996 / AF393847	4,408-4,423	16	Sequence used for DNA cloning
14. *pAgp*, 2nd copy	*S. tuberosum* var. Ranger Russet	HM363752	4,424-6,683	2260	Drives expression of PhL and R1 inverted repeats , especially in tubers
15. Fragment of the region extending from the 5'-untranslated region into the promoter of the potato phosphorylase-L (*PhL*) gene (1st copy, antisense orientation)	*S. tuberosum* var. Ranger Russet	HM363758	6,684-7,192	509	Generates dsRNA that triggers the degradation of *PhL* transcripts to limit the formation of reducing sugars
16. Fragment of promoter for the potato *R1* gene (1st copy, antisense orientation)	*S. tuberosum* var. Ranger Russet	HM363757	7,193-7,724	532	Generates dsRNA that triggers the degradation of *R1* transcripts to limit the formation of reducing sugars
17. Intervening Sequence	*S. tuberosum*	DQ478950	7,725-7,730	6	Sequence used for DNA cloning
18. Spacer-2	*S. tuberosum* var. Ranger Russet	U26831[4]	7,731-7,988	258	Sequence between the 2nd inverted repeat; transcript forms loop in dsRNA
19. Fragment of promoter for the potato *R1* gene (2nd copy, sense orientation)	*S. tuberosum* var. Ranger Russet	HM363757	7,989-8,520	532	Generates dsRNA that triggers the degradation of *R1* transcripts to limit the formation of reducing sugars

20. Fragment of the region extending from the 5'-untranslated region into the promoter of the potato phosphorylase-L (*PhL*) gene (2nd copy, sense orientation)	*S. tuberosum* var. Ranger Russet	HM363758	8,521-9,029	509	Generates dsRNA that triggers the degradation of *PhL* transcript to limit the formation of reducing sugars
21. *pGbss* (2nd copy, opposite direction from 2nd copy of *pAgp*)	*S. tuberosum* var. Ranger Russet	X83220[5]	9,030-9,953	924	Drives expression of PhL and R1 repeats, especially in tubers
22. Intervening Sequence	*S. tuberosum*	AF143202	9,954 – 9,962	9	Sequence used for DNA cloning
23. Right Border (RB) region sequence	*S. tuberosum* var. Ranger Russet	AY566555[2]	9,963 – 10,123	161	Buffer for truncations during insertion
24. RB sequence[1]	Synthetic	AY566555[2]	10,124 – 10,148	25	Primary cleavage site releases ssDNA insert from pSIM1278 (van Haaren et al., 1989)

[1]The LB and RB sequences (25-bp each) were synthetically designed to be similar to and function like T-DNA borders from *Agrobacterium tumefaciens*.
[2]GenBank Accession AY566555 was revised to clarify the sources of DNA for the border regions.
[3]ASN1 described as genetic elements 5 and 10 is referred to as StAst1 in Chawla et al., 2012.
[4]GenBank Accession HM363756 is replaced with a citation to GenBank Accession U26831 to include four 3' end nucleotides present in the pGbss DNA element of the pSIM1278 construct.
[5]GenBank Accession HM363755 is replaced with a citation to GenBank Accession X83220 to include the full *pGbss* (2nd copy) DNA insert sequence present in the pSIM1278 construct.

Table 4. Genetic Elements of pSIM1678, from Left Border to Right Border

Genetic Element	*Origin*	Accession Number	Position (pSIM1678)	Size (bp)	Intended Function
1. Left Border (LB) site[1]	Synthetic	AY566555[3]	1–25	25	Secondary cleavage site releases ssDNA insert from pSIM1678 (van Haaren et al., 1989)
2. LB region	*S. tuberosum* var. Ranger Russet	AY566555[3]	26-187	162	Buffer for truncations during insertion
3. Intervening Sequence	*S. tuberosum*	AF393847	188–193	6	Sequence used for DNA cloning
4. Native *Rpi-vnt1* gene promoter	*S. venturii*	FJ423044	194 -902	709	Drives expression of the *Rpi-vnt1* gene
5. *Rpi-vnt1* gene coding sequence	*S. venturii*	FJ423044	903 – 3,578	2676	Expresses the VNT1 protein for late blight protection
6. Native *Rpi-vnt1* gene terminator	*S. venturii*	FJ423044	3,579 – 4,503	925	Terminates transcription of *Rpi-vnt1*
7. Intervening Sequence	*S. tuberosum*	HM363755	4,504 - 4,510	7	Sequence used for DNA cloning
8. ADP glucose pyrophosphorylase gene promoter (*pAgp*)	*S. tuberosum* var. Ranger Russet	HM363752	4,511 - 6,770	2260	Drives expression of the *VInv* inverted repeat, especially in tubers
9. Intervening Sequence	*S. tuberosum* var. Ranger Russet	DQ206630	6,771 - 6,776	6	Sequence used for DNA cloning
10. *VInv* gene fragment (sense orientation)	*S. tuberosum* var. Ranger Russet	DQ478950	6,777 – 7,274	498	Generates dsRNA to down regulate *VInv* transcripts
11. *VInv* gene fragment (sense orientation)	*S. tuberosum* var. Ranger Russet	DQ478950	7,275 – 7,455	181	Sequence between the inverted repeat; transcript forms loop in dsRNA
12. Intervening Sequence	*S. tuberosum* var. Ranger Russet	X73477	7,456 - 7,461	6	Sequence used for DNA cloning
13. *VInv* gene fragment (anti-sense orientation)	*S. tuberosum* var. Ranger Russet	DQ478950	7,462 - 7,959	498	Generates dsRNA to down regulate *VInv* transcripts
14. Intervening Sequence	*S. tuberosum* var. Ranger Russet	X95996	7,960 - 7,971	12	Sequence used for DNA cloning
15. Granule-bound starch synthase gene promoter (*pGbss*) (opposite direction from *pAgp*)	*S. tuberosum* var. Ranger Russet	X83220[2]	7,972 - 8,894	923	Drives expression of the *VInv* inverted repeat, especially in tubers
16. Intervening Sequence	*S. tuberosum*	AF143202	8,895 – 8,903	9	Sequence used for DNA cloning
17. Right Border (RB) region	*S. tuberosum* var. Ranger Russet	AY566555[3]	8,904– 9,064	161	Buffer for truncations during insertion
18. RB sequence[1]	Synthetic	AY566555[3]	9,065 – 9,089	25	Primary cleavage releases ssDNA insert from pSIM1678 (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 HM363755 is replaced with a citation to GenBank Accession X83220 to include the full *pGbss* (2nd copy) DNA insert sequence present in the pSIM1278 construct.
[3]GenBank Accession AY566555 was revised to clarify the sources of DNA for the Border regions

A.2(a)(ii) History of use of the organism in the food supply or history of human exposure to the organism through other than intended food use (e.g. as a normal contaminant)

The potato is the world's fourth largest food crop, following rice, wheat, and maize. It has a long history in the diets of humans across the entire world.

The Inca Indians in Peru were the first to cultivate potatoes around 8,000 BC to 5,000 BC.

In 1536 Spanish Conquistadors conquered Peru, discovered the flavours of the potato, and carried them to Europe. Before the end of the sixteenth century, families of Basque sailors began to cultivate potatoes along the Biscay coast of northern Spain. Sir Walter Raleigh introduced potatoes to Ireland in 1589 on the 40,000 acres of land near Cork. It took four decades for the potato to spread to the rest of Europe.

Potatoes arrived in the Colonies in 1621 and the first permanent potato patches in North America were established in 1719. From there, the crop spread across the United States.

Potatoes were introduced into Australia with the early European settlers. In 1797, Governor Hunter reported that 11 acres (4.5 (ha)) were under potato crop in the Parramatta district west of Sydney. A decade later, this area had increased to 301 acres (122 ha); and nearly a century later in 1906, 119,000 acres (48,000 ha) of potatoes were under crop in Australia.

Today, potato production occurs around Australia with the exception of the far northern areas where temperatures exceed the optimal growing conditions for this cool-season crop. All states grow significant quantities of potatoes with predominant production in the cooler states of South Australia, Tasmania and Victoria.

Sources: International Year of the Potato (FAO, 2008); Feature Article: Potaotes – The world's favourite vegetable (Year Book Australia 2008, Australian Bureau of Statistics, 2008)

A.2(b) A description of the host organism into which the genes were transferred:

A.2(b)(i) Its history of safe use for food

See Section A.2(a) (ii).

A.2(b)(ii) The part of the organism typically used as food

Potato tubers are the only part consumed as food.

A.2(b)(iii) The types of products likely to include the food or food ingredient

In Australia and New Zealand, consumer research has confirmed that all major uses of potato involve some level of cooked preparation. The versatility of potato has led to a range of uses, with the majority being centred around dinner, and to lesser degree lunch time occasions. Seasonal influences have little bearing on the main uses of potato, reflecting patterns of consistent consumption year-round. Within the Australian and New Zealand diet, potatoes are prepared and consumed in the following forms:

- Boiled, Microwaved, Steamed
- Deep fried
- Mashed
- Roasted
- Baked/Grilled

- Salad – cooked
- Soup/Sauce
- Stir fry
- Juiced
- Sandwich/burger/wrap
- Snacks – potato chips / straws.

A.2(b)(iv) Whether special processing is required to render food derived from the organism safe to eat

Potato tubers for direct consumption should be cooked before eating because of the indigestibility of non-gelatinised starch and the presence of anti-nutritional proteins (OECD, 2002).

Potatoes are prepared and packaged fresh as well as processed for fries, chips and flakes.

Potatoes are prepared in many ways: skin-on or peeled, whole or cut up, with seasonings or without. The only requirement involves cooking to swell the starch granules. Most potato dishes are served hot, but some are first cooked, then served cold, notably potato salad and potato chips/crisps.

Other uses include:

- Used to brew alcoholic beverages such as vodka, potcheen, or akvavit
- Feed for domestic animals
- Potato starch is used in the food industry as, for example, thickeners and binders of soups and sauces, in the textile industry, as adhesives, and for the manufacturing of papers and boards
- Potato skins, along with honey, are a folk remedy for burns in India. Burn centres in India have experimented with the use of the thin outer skin layer to protect burns while healing.

A.3. The nature of the genetic modification

A.3(a) A description of the method used to transform the host organism

The W8, X17, and Y9 events were developed by first transforming Russet Burbank, Ranger Russet, and Atlantic, respectively, with the plasmid pSIM1278 to reduce expression of asparagine synthetase, polyphenol oxidase, and the starch-associated enzymes, water dikinase and phosphorylase L.

In order to reduce expression of the vacuolar invertase transcripts (*VInv*) and add the late blight protection gene (*Rpi-vnt1*), a second transformation was carried out with plasmid pSIM1678. These plasmids are described in detail in Table 3 and Table 4 and Section A3(b). A synopsis of the different events is provided in Table 5.

Table 5. Synopsis of Simplot Biotech Potato Events

Product category	Plasmids Used for Transformation	Variety			
		Russet Burbank		Ranger Russet	Atlantic
Gen1	pSIM1278	E12	E56	F10	J3
Gen2	Gen1 + pSIM1678	none	**W8***	**X17**	**Y9**

*W8 was developed from E56, which was not commercialised.

The pSIM1278 and pSIM1678 transformation protocols are described in Figure 1 and Figure 2.

Somaclonal variation occurs when genetically dissimilar individuals are derived from vegetative propagation. *S. tuberosum* varieties are prone to somaclonal variation during the callus stage that occurs during transformation and may exhibit a degree of heterogeneity (OECD, 1997). Several steps were taken during event selection to mitigate somaclonal variation. These steps were:

1. A number of transformed plants were produced;
2. A late blight assay was used to screen for plants expressing the VNT1 protein;
3. Plants exhibiting partial protection against late blight were discarded;
4. Asymptomatic plants were selected and advanced for field testing; and
5. Any plants with off-types were removed.

The selection process was conducted by studying the growth characteristics of the transformed events compared to controls. Field trials evaluating phenotypic and agronomic characteristics did not identify any somaclonal variation in W8, X17, and Y9.

Conclusion of the Development of W8, X17, and Y9

Events W8, X17, and Y9 were developed by transforming the Russet Burbank, Ranger Russet, and Atlantic varieties, respectively, with pSIM1278 and then retransforming with pSIM1678. Transformation of each variety introduced DNA sequence (*Asn1*, *R1*, *Ppo5*, *PhL*, and *VInv*) intended to down regulate asparagine synthetase, polyphenol oxidase, water dikinase, phosphorylase, and vacuolar invertase through the mechanism of RNAi. In addition, the late blight resistance gene, *Rpi-vnt1,* under the control of its native promoter and terminator was introduced.

All genetic elements between the LB and RB of the T-DNA in pSIM1278 and pSIM1678 were derived from the genomes of cultivated and wild solanum species. Several steps were taken throughout the transformation and selection process to discard any plants showing somaclonal variation or the presence of backbone sequence.

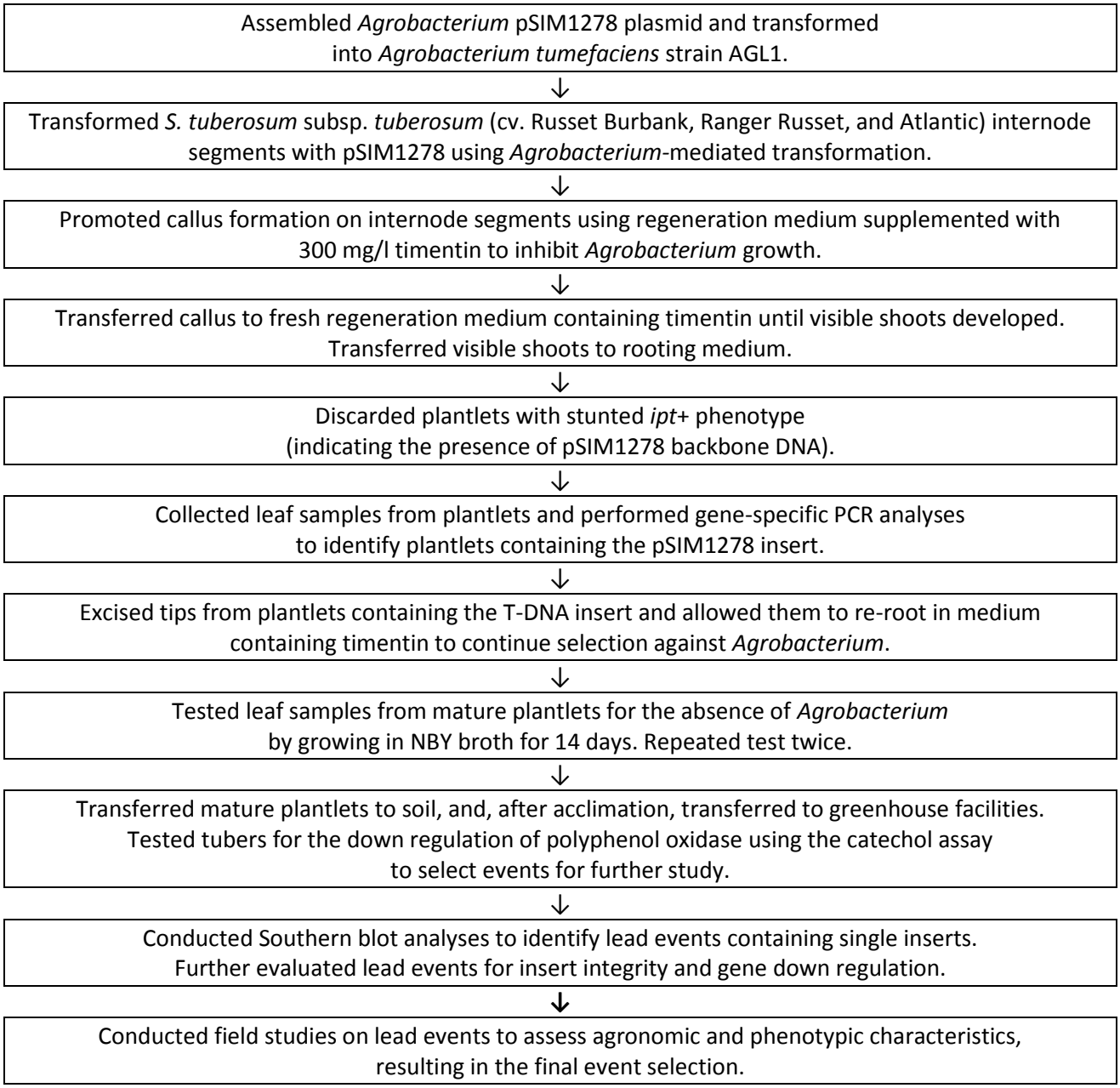


Figure 1. The Development and Selection of Events Transformed with pSIM1278

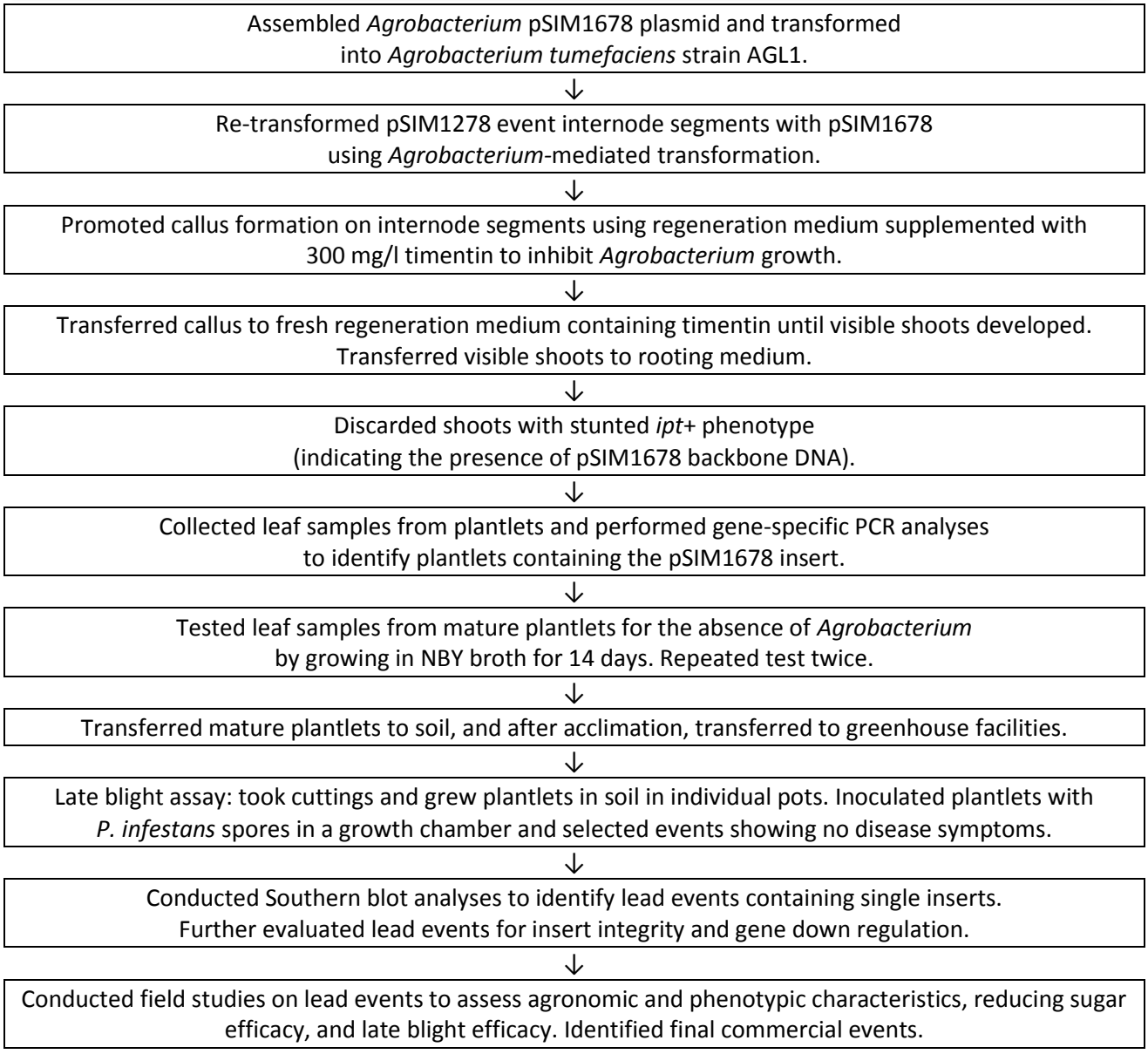


Figure 2. Development and Selection of W8, X17, and Y9 Transformed with pSIM1678

A.3(b) A description of the construct and the transformation vectors used

The events W8, X17, and Y9 were developed by transforming the Russet Burbank, Ranger Russet, and Atlantic varieties, respectively, with pSIM1278 followed by transformation with pSIM1678. All genetic elements between the left border (LB) and right border (RB) of the T-DNA in pSIM1278 and pSIM1678, were derived from the genomes of cultivated and wild potato species. The resulting varieties possess late blight protection and exhibit a reduction in black spot, reducing sugars, and acrylamide potential.

The pSIM1278 and pSIM1678 plasmids were constructed using the same parental plasmid and therefore share identical backbone sequences (Table 6). The backbone contains two well-characterised bacterial origins of replication:

- pVS1 (pVS1 Sta and Rep), which enables maintenance of the plasmid in *Agrobacterium* and
- pBR322 (pBR322 bom and ori), which enables maintenance of the plasmid in *Escherichia coli*.

Additional backbone elements include:

- The *Agrobacterium* DNA overdrive sequence enhances cleavage at the RB;
- The kanamycin resistance gene (Kan^R) functions as a selectable marker for maintenance in bacteria; and
- The *Agrobacterium* isopentenyl transferase (*ipt*) gene flanked by the Ranger Russet potato polyubiquitin (*Ubi7*) promoter and the Ranger Russet potato polyubiquitin (*Ubi3*) terminator (Garbarino and Belknap, 1994).

The backbone contains a cassette comprising the *Agrobacterium* isopentenyl transferase (*ipt*) gene flanked by the Ranger Russet potato polyubiquitin (*Ubi7*) promoter and the Ranger Russet potato polyubiquitin (*Ubi3*) terminator (Garbarino and Belknap, 1994). Although *Agrobacterium* is effective in cleaving at the RB aided by the overdrive sequence, cleavage at the LB is often less precise (Gelvin, 2003), resulting of the transfer of backbone sequence in addition to the T-DNA.

Overexpression of *ipt* was used as a screenable phenotype to select against the integration of plasmid backbone DNA (Table 6). When present in transformed plants, overexpression of *ipt* results in the overproduction of the plant hormone, cytokinin. This causes plants with stunted phenotypes, abnormal leaves and the inability to form roots. Only plantlets that were phenotypically indistinguishable from non-transformed controls were selected and allowed to develop. This screening ensured that transformed plants lacked the functionally active backbone *ipt* marker gene (Richael et al., 2008).

Maps of pSIM1278 and pSIM1678, are provided in Figure 3 and Figure 4, respectively, with corresponding descriptions of the genetic elements in the T-DNA provided in Table 3 and Table 4.

The plasmid, pSIM1278, is a 19.6 kb binary transformation vector used in the first transformation of Russet Burbank, Ranger Russet, and Atlantic.

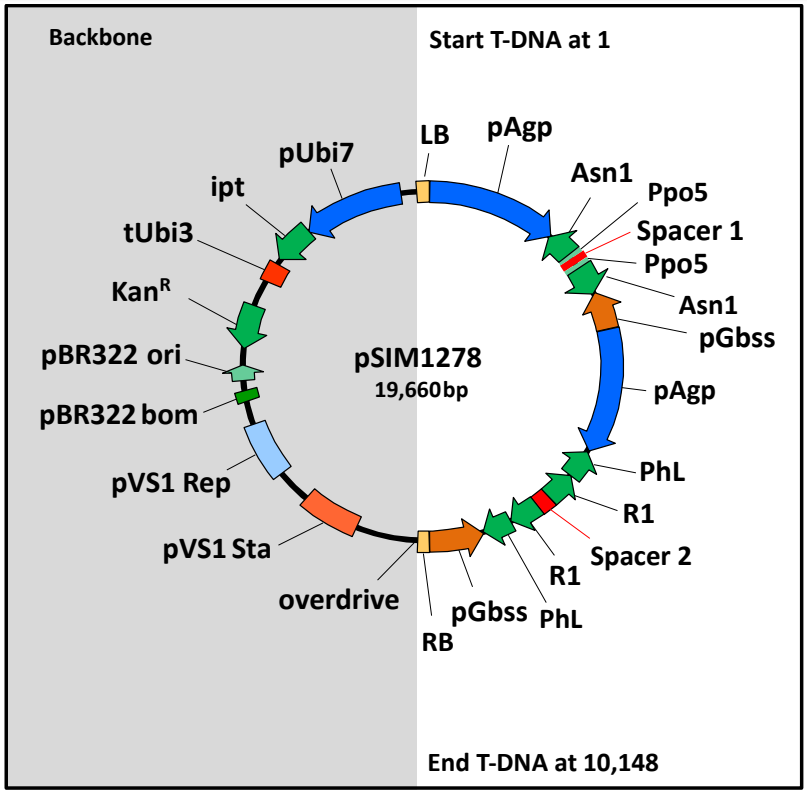


Figure 3. Plasmid Map of pSIM1278

The backbone region (grey background) starts at position 10,149 bp and ends at 19,660 bp. The backbone consists of bacterial and potato DNA and is intended only to support maintenance of the DNA insert prior to plant transformation. The T-DNA insert region (white background) is from 1 bp to 10,148 bp.

pSIM1278 T-DNA contains two down-regulation cassettes (Figure 3):

- The first (elements 4 to 12; Table 3) is designed to down regulate asparagine synthetase and polyphenol oxidase in the transformed potato variety. The inverted repeat is comprised of asparagine synthetase 1 (*Asn1*) and polyphenol oxidase 5 (*Ppo5*) fragments, separated by a spacer element (Spacer-1) and arranged between two convergent potato promoters: the *Agp* promoter of the ADP glucose pyrophosphorylase gene (*Agp*), and the *Gbss* promoter of the granule-bound starch synthase gene (*Gbss*). Both promoters are primarily active in tubers
- The second (elements 14 to 21; Table 3) is designed to down regulate phosphorylase L and water dikinase in the transformed potato variety. The inverted repeat is comprised of phosphorylase L (*PhL*) and water dikinase (*R1*) fragments, separated by a spacer element (Spacer-2) and arranged between the two convergent potato promoters, *Agp* and *Gbss*.

The plasmid, pSIM1678, is an 18.6 kb binary transformation vector. A plasmid map for pSIM1678 is provided in Figure 4. As mentioned above, the plasmids were constructed using the same parental plasmid and therefore share identical backbone sequences (Table 6).

pSIM1678 T-DNA contains the *Rpi-vnt1* cassette and a down-regulation cassette (Figure 4):

- The *Rpi-vnt1* cassette (elements 4 to 6; Table 4) contains the 2,676 bp *Rpi-vnt1* gene. The gene is expressed under the native promoter (*pVnt1*) and terminator (*tVnt1*) and
- The VInv down-regulation cassette, (elements 8 to 15; Table 4) is designed to down regulate vacuolar invertase (VINV) in the transformed potato variety. The inverted repeat is comprised of

VInv fragments, separated by a spacer element (also a *VInv* fragment) and arranged between the two convergent potato promoters, *Agp* and *Gbss*.

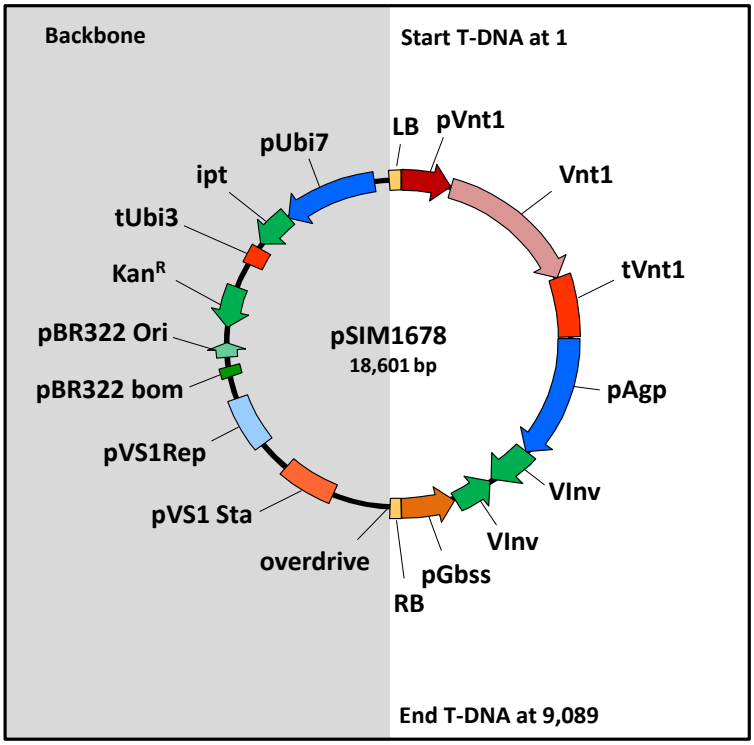


Figure 4.Plasmid Map of pSIM1678

The backbone region (grey background) starts at position 9,090 bp and ends at 18,601 bp. The backbone DNA is the same between plasmids pSIM1278 and pSIM1678. The backbone consists of bacterial and potato DNA and is intended only to support maintenance of the DNA insert prior to plant transformation. The T-DNA insert region (white background) is from 1 bp to 9,089 bp.

Table 6. Genetic Elements of the pSIM1278 and pSIM1678 Plasmid Backbone

Genetic Element	Origin	Accession Number[1]	Position for pSMI1278[1]	Size (bp)	Function
1. Intervening sequence	Synthetic DNA		10,149-10,154	6	Sequence used for cloning
2. Overdrive	*Agrobacterium tumefaciens* Ti-plasmid	NC_002377	10,155-10,184	30	Enhances cleavage at *A. tumefaciens* Right Border site [1]
3. Intervening sequence	*Pseudomonas fluorescens* pVS1	AJ537514	10,185-11,266	1,082	pVS1 backbone[1]
4. pVS1 partitioning protein StaA (PVS1 Sta)	*P. fluorescens* pVS1	AJ537514	11,267-12,267	1,001	pVS1 stability [1]
5. Intervening sequence	*P. fluorescens* pVS1	AJ537514	12,268-12,860	593	pVS1 backbone[1]
6. pVS1 replicon (pVS1Rep)	*P. fluorescens* pVS1	AJ537514	12,861-13,861	1,001	pVS1 replication region in *Agrobacterium* [1]
7. Intervening sequence	*P. fluorescens* pVS1	AJ537514	13,862-14,099	238	pVS1 backbone[1]
8. Intervening sequence	pBR322	AF234297	14,100-14,270	171	pCambia1301 backbone[1]
9. pBR322 bom	pBR322	AF234297	14,271-14,531	261	pBR322 region for replication in *E. coli* [1]
10. Intervening sequence	pBR322	AF234297	14,532-14,670	139	pCambia1301 backbone[1]
11. Origin of replication for pBR322 (pBR322 ori)	pBR322	AF234297	14,671-14,951	281	Bacterial origin of replication [1]
12. Intervening sequence	pBR322	AF234297	14,952-15,241	290	pCambia1301 backbone[1]
13. Aminoglycoside phosphotransferase gene	pCambia1301	AF234297	15,242-16,036	795	Kanamycin resistance gene[1]
14. Intervening sequence	Vector DNA	FJ362602	16,037-16,231	195	pCambia1301 vector backbone[1]
15. Terminator of the ubiquitin-3 gene (*tUbi3*)	*S. tuberosum*	GP755544	16,232-16,586	355	Terminator for *ipt* gene transcription (Garbarino and Belknap, 1994)
16. Intervening sequence	*A. tumefaciens* Ti-plasmid	NC_002377	16,587-16,937	351	Sequence used for DNA cloning
17. Isopentenyl transferase (*ipt*) gene	*A. tumefaciens* Ti-plasmid	NC_002377	16,938-17,660	723	Condensation of AMP and isopentenyl-pyrophosphate to form isopentenyl-AMP, a cytokinin in the plant. Results in abnormal growth phenotypes in plant (Smigocki and

					Owens, 1988)
18. Intervening sequence	Synthetic DNA		17,661-17,672	12	Sequence used for DNA cloning
19. Polyubiquitin promoter (*pUbi7*)	*S. tuberosum* var. Ranger Russet	U26831	17,673-19,410	1,738	Promoter to drive expression of the *ipt* backbone marker gene (Garbarino et al., 1995)
20. Intervening sequence	Vector DNA	U10460	19,411-19,660	250	pZP200 vector backbone[1]

[1] Position numbers for pSIM1678 are different, but the elements are the same

Potato Genes Targeted for Down Regulation using RNAi

Transcription of the inverted repeats leads to down regulation of endogenous target genes through production of dsRNA and the plant's RNAi pathway. The inverted repeats are derived from the DNA sequences of five target potato genes (*Asn1*, *Ppo5*, *PhL*, *R1,* and *VInv*; Table 2).

The T-DNA in each plasmid contains down-regulation cassettes that result in the production of siRNA in W8, X17, and Y9 using the plant's RNAi pathway. As described above, each down-regulation cassette is comprised of DNA sequence arranged as an inverted repeat.

Due to the nature of the inverted repeat sequences, their transcripts form dsRNA through complementary binding. The dsRNA act as a precursor for the plant's own RNAi post-transcriptional regulatory pathway. A cellular RNase III enzyme, Dicer, recognises and processes the precursor dsRNA into 21-24 bp duplexes termed siRNA. The siRNA bind with cellular proteins forming RNA Induced Silencing Complexes (RISC). The RISC selectively degrades one of the siRNA strands, referred to as the passenger strand. The remaining strand, referred to as the guide strand, targets the complementary sequence in an mRNA molecule. Once the guide strand pairs with an mRNA in a RISC complex, the mRNA molecule is cleaved and degraded, preventing protein translation (Chau and Lee, 2007; Fire et al., 1998).

Gene for Late Blight Protection

The *Rpi-vnt1* gene found in *S. venturii* and *S. phureja* confers protection against the oomycete that causes late blight disease in potatoes, *P. infestans*. Late blight protection was achieved in W8, X17, and Y9 by the addition of the *Rpi-vnt1* gene with its native promoter and termination sequence. The *Rpi-vnt1* gene was cloned from a wild *Solanum* species, *S. venturii,* and is identical to the *Rpi-phu1* gene found in *S. phureja* (Śliwka et al., 2013).

A.3(c) A full molecular characterisation of the genetic modification in the new organism

The molecular characterisation of Events W8, X17, and Y9 was performed using:

- Southern, PCR, and sequencing analyses showing the structure of the inserts
- Southern analysis confirming the absence of backbone sequence
- Southern analysis showing each insert integrated into a single genomic locus
- Analysis of the junction regions with the host DNA
- Mapping showing the organisation of the inserted genetic material at each insertion site
- Details of an analysis of the insert junction regions for the occurrence of potential open reading frames (ORFs).

Full details describing the characterisation, including materials and methods used in these studies are provided in **SUPPLEMENT 1** and the Reports:

- Report15-67-SPS-MOL
- Report15-04-SPS-MOL
- Report15-12-SPS-MOL

Further details of molecular characterisation are provided in this Section, including:

- Southern analysis confirming stability of the inserts (see Section A3(e)) and Report15-37-SPS-MOL, Report15-06-SPS-MOL, Report15-14-SPS-MOL
- An analysis of expressed RNA transcripts, where RNA interference has been used (see Section A3(g)).

Summary of molecular characterisation

The molecular characterisation of W8 demonstrated the following:

- Each insert is integrated at a single locus;
- The pSIM1278 insert contains a nearly full-length T-DNA flanked by a duplication of the Asn1/Ppo5 cassette on the left side and an additional PhL/R1 cassette on the right side and includes a duplication of the *Gbss* promoter with intervening *PhL* sequence;
- The pSIM1678 insert is similar to the pSIM1678 T-DNA;
- There is no backbone sequence present in W8;
- PCR followed by Sanger sequencing, Illumina Hi-Seq, or PacBio Single Molecule Real Time Sequencing (SMRT) was used to identify the unique junctions within the pSIM1278 insert and the site of integration for each insert within the W8 genome. Alignment of the pSIM1278 integration site sequence to the Michigan State University (MSU) potato reference genome (Xu et al., 2011) indicates the likely integration site is on chromosome 2, where no gene is present;

- Alignment of the pSIM1678 integration site to the Spud Database indicates integration on chromosome 4, though exact coordinates of integration could not be determined due to differences between the MSU potato reference genome assembly and the Russet Burbank genome;
- There are no ORFs covering the left or right junctions associated with the pSIM1278 insert in W8;
- There are no ORFs covering the right junction associated with the pSIM1678 insert in W8. Two ORFs, in different reading frames, span the left junction. None of the ORFs were identified as homologs of known toxins or allergens; and
- Each insert is shown to be stable during vegetative propagation.

The molecular characterisation of X17 demonstrated the following:

- Each X17 insert is integrated at a single locus;
- The pSIM1278 insert consists of a nearly full-length T-DNA;
- The pSIM1678 insert consists of a nearly full-length T-DNA;
- There is no backbone sequence present in X17;
- PCR followed by Sanger sequencing was used to identify the unique junctions at the boundaries between the X17 inserts and the potato genomic DNA. Additionally, the sites of integration in the potato genome were amplified and sequenced. Alignment of the integration site sequences to the MSU potato reference genome indicates the likely integration site is on chromosome 8 for pSIM1278 and chromosome 5 for pSIM1678. Both insertions occurred at a locus containing an annotated potato gene;
- There are no ORFs covering the left junction associated with the pSIM1278 insert in X17. A single ORF was identified spanning the right junction. It is almost completely native potato sequence;
- There are no ORFs covering the left junction associated with the pSIM1678 insert in X17. A single ORF was identified spanning the right junction. It is almost completely native potato sequence;
- None of the X17 junction ORFs were identified as homologs of known toxins or allergens; and
- Each X17 insert is shown to be stable during vegetative propagation.

The molecular characterisation of Y9 demonstrated the following:

- Each Y9 insert is integrated at a single locus;
- The pSIM1278 insert consists of a nearly full-length T-DNA flanked by an additional Asn1/Ppo5 and partial PhL/R1 cassette;
- The pSIM1678 insert consists of a nearly full-length T-DNA flanked by an additional, partial VInv cassette;
- There is no backbone sequence present in Y9;

- Sanger or Illumina Hi-Seq sequencing of PCR amplicons was used to identify the sites of integration for each insert within the Y9 genome and unique junctions for each insert. Alignment to the MSU potato reference genome indicated a likely integration site for the pSIM1278 insert on chromosome 6 that did not disrupt any known genes. The pSIM1678 integration site was identified on chromosome 5. Unique chromosome coordinates were not determined because of differences between the Atlantic genome and MSU potato reference genome;
- There are no ORFs covering the left junction associated with the pSIM1278 insert in Y9. A single ORF was identified spanning the left junction;
- There are no ORFs covering the right junction associated with the pSIM1678 insert in Y9. A single ORF was identified spanning the left junction;
- None of the Y9 junction ORFs were identified as homologs of known toxins or allergens; and
- Each Y9 insert is shown to be stable during vegetative propagation.

A.3(d) A description of how the line or strain from which food is derived was obtained from the original transformant (i.e. provide a family tree or describe the breeding process) including which generations have been used.

Potato plants are cultivated by vegetative propagation and commonly maintained as disease-free plantlets in tissue culture. Shoots from the plantlets are cut and transferred to fresh medium periodically to maintain healthy stocks (Figure 5). When many plants are needed, for example for seed production, multiple shoots are cut and grown in tissue culture medium. Plantlets with roots are transferred to greenhouses to produce tubers for seed. Greenhouse tubers are planted in fields to multiply potato seed for large-scale potato production.

Potato seed is a tuber that contains buds, called “eyes”, which sprout and grow into mature potato plants. The seed tuber is planted whole or as a cut piece with eyes (Figure 5).

In vegetative (asexual) propagation, progeny arise from a single parent plant, and each progeny cell receives the same genetic material. As the parent cell divides in two, the resulting progeny cells are copies of one another and the parent plant. Vegetative propagation produces a genetically uniform crop because no new genetic material is introduced through sexual reproduction.

During seed production growers eliminate plants in the field that are “off-types” in order to maintain the desired characteristics of the variety. In addition, commercial seed is reinitiated from the disease-free, tissue culture source material on a continuous basis.

As a result of vegetative propagation, each potato is a genetic clone of its parent plant since the tuber (and not the true seed) is used to generate the next plants (Figure 6). An example of potato vegetative propagation is shown in (Figure 6). Tuber production begins with parent plants containing the desired traits of interest. Cuttings from the parent plants are propagated in tissue culture (plantlets). These are transferred to greenhouses to grow mini-tubers for distribution to seed farmers. Mini-tubers are planted in fields to produce tuber seed. After multiple seasons of re-planting tubers (generally 3–5

seasons), the tubers will be sold for commercial potato production and seed production reverts to tissue culture parent plants that have been tested to confirm the presence of the desired traits and disease free status.

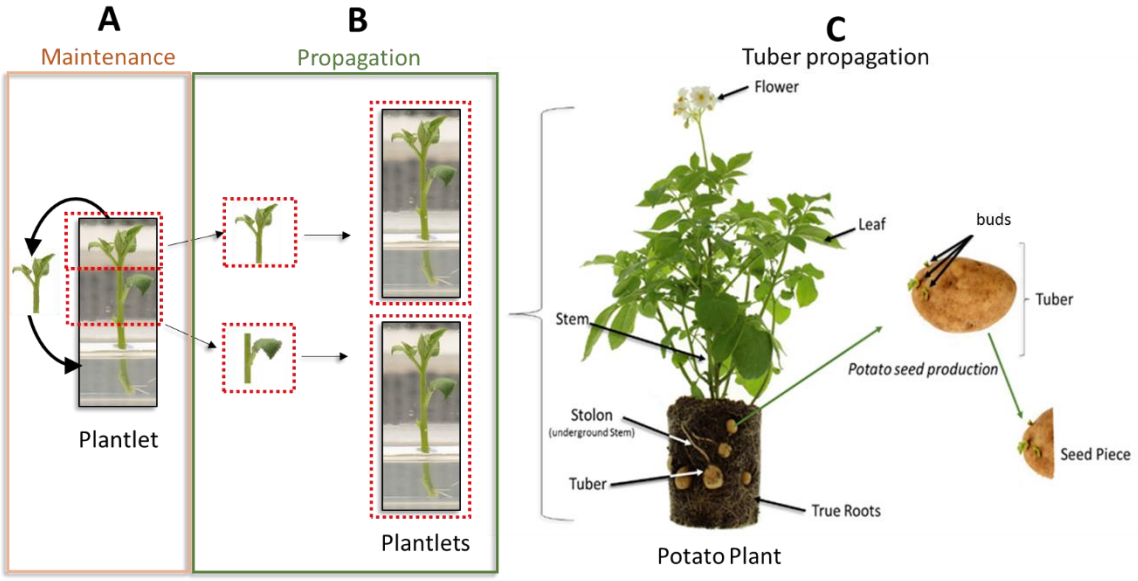


Figure 5. Potato Plant and Tuber Propagation

Potato variety stocks are maintained in tissue culture (A) and multiplied by vegetative propagation (B) to produce mature potato plants and potato seed (C).

Figure 6. Commercial Production of Potatoes

Plantlets are propagated from cuttings of the stock tissue culture plantlet. Plantlets are transferred to soil or grown using Nutrient Film Technique (NFT) or hydroponics in greenhouses. Tubers from these Greenhouse-0 plants are referred to as mini-tubers. An entire mini-tuber is planted in either the greenhouse or field to produce a new potato plant. Tubers from Greenhouse-1/Field Grown-1 plants can be cut into 2-4 oz. (55-115 g) pieces, which contain lateral buds, and are used as “seed pieces” to produce Greenhouse-2/Field Grown-2 plants. The process of vegetative propagation is repeated to generate planting material.

A.3(e) Evidence of the stability of the genetic changes, including:

(i) The pattern of inheritance of the transferred gene(s) and the number of generations over which this has been monitored

(ii) The pattern of inheritance and expression of the phenotype over several generations and, where appropriate, across different environments

Because commercial potatoes are vegetatively propagated, the progeny from a parent cell are genetically identical to each other and the parent plant (Section A3(d)). Therefore, the T-DNA inserts in W8, X17, and Y9 are expected to be genetically stable during vegetative propagation. Consequently, evaluating insert stability by examining inheritance using Mendelian segregation analysis is not applicable for potatoes. Nonetheless, stability of inserted DNA in W8, X17, and Y9 was examined across multiple vegetative propagations using Southern blot analysis. Methods for this investigation are provided in Appendix A in Supplement 1.

Southern blot analyses were conducted to verify the stability of the two DNA inserts in event W8 (Report 15-37-SPS-MOL). Stability of the pSIM1278 and pSIM1678 inserts were assessed in potato plants at different time points. Plants were grown in the greenhouse in Sunshine mix-1 (www.sungro.com) in two-gallon pots controlled for temperature (18 °C minimum/27 °C maximum) and light (16-h photoperiod with an intensity of about 1,500 µmol/m2/s). Sprouted mini-tubers (G0) were planted to produce G1 plants (Figure 7). G1 plants were grown until maturity to produce G1 tubers that were planted to produce G2 plants. Samples were collected from Russet Burbank and W8 transformed plants (G0 and G2) for Southern blot analysis.

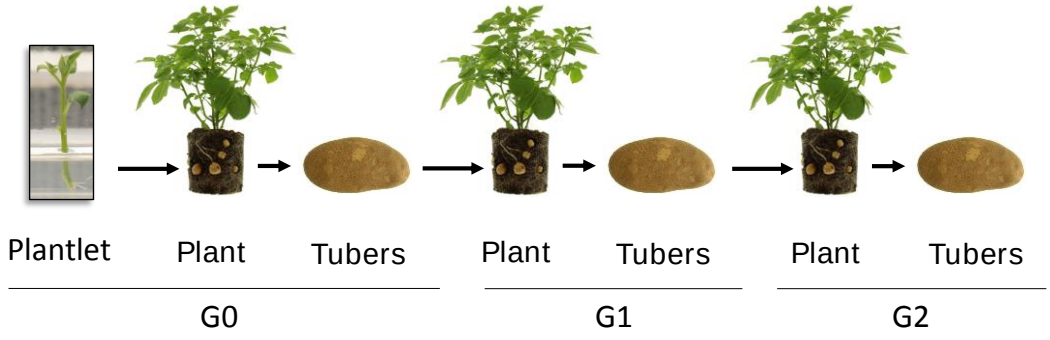


Figure 7. Vegetative Propagation of Potato Plants

Tissue culture plantlets were planted in soil to produce tubers, designated G0. G0 tubers were planted to produce G1 plants and tubers, which were used to produce G2 plants and tubers. DNA was isolated from G0 and G2 samples of vegetatively-propagated greenhouse plants for Southern blot analysis.

Stability of Event W8

W8 and Russet Burbank DNA was digested with EcoRV and XbaI restriction enzymes and hybridised with AGP, GBS1, VNT and INV probes, respectively (Figure 8). Evidence of genetic stability was indicated based upon consistent banding patterns between G0 and G2 samples. The Southern blot bands unique to W8 are associated with the inserts, whereas endogenous bands are found in both Russet Burbank and W8 samples.

The structures of the pSIM1278 and pSIM1678 inserts are depicted in Figure 8. The expected restriction digestion products are indicated below for each restriction enzyme used along with the fragment sizes and probe hybridisation specificity.

The AGP probe hybridises to restriction fragments associated with both of the pSIM1278 (2.3 and 10 kb) and pSIM1678 (7 kb) inserts. All five fragments were detected in the G0 and G2 samples for W8 (Figure 9A). The 10 and 7 kb fragments correspond to the left sides of the pSIM1278 and pSIM1678 inserts, whereas the three 2.3 kb fragments are located within the pSIM1278 insert. As expected, increased band intensity was observed for the 2.3 kb fragment since the pSIM1278 insert contains three copies of this region (Figure 8A).

Similarly, the GBS probe hybridises to fragments associated with both inserts, pSIM1278 (2.3 kb and 14 kb) and pSIM1678 (3.6 kb). The GBS probe hybridises to the right end of the insert where fragments of 14 kb (pSIM1278) and 3.6 kb (pSIM1678) were observed (Figure 9B). Again, increased band intensity was associated with the 2.3 kb internal fragment due to multiple copies within the pSIM1278 insert.

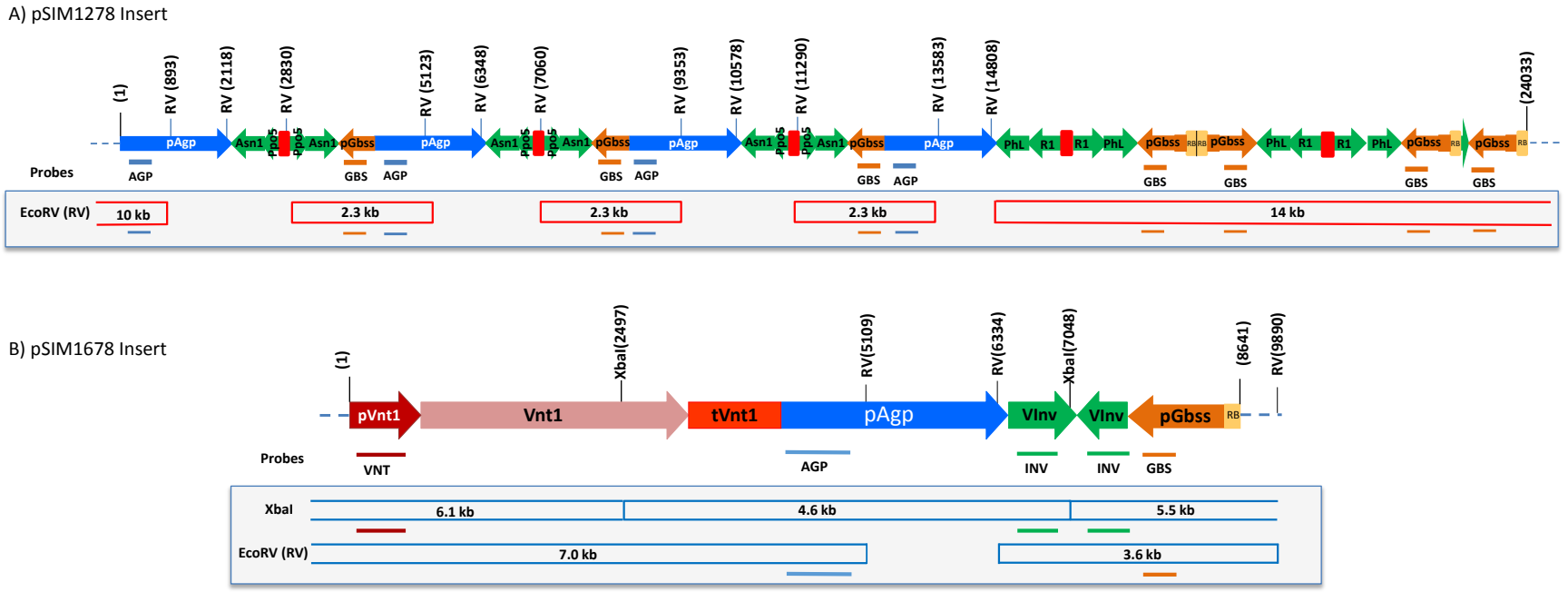


Figure 8. Structures of the pSIM1278 and pSIM1678 DNA Inserts in W8

(A) The structure of the pSIM1278 insert is depicted along with EcoRV restriction sites. The expected digestion products following an EcoRV digest are depicted below in the grey box along with the fragment size and hybridisation sites for AGP and GBS probes. (B) The structure of the pSIM1678 insert is depicted along with EcoRV and XbaI restriction sites. The expected digestion products following an XbaI or EcoRV digest are depicted below in the grey box along with the fragment size and hybridisation sites for VNT, INV, AGP and GBS probes. Red boxes are fragments associated with pSIM1278. Blue boxes are fragments associated with pSIM1678. A coloured line indicates the binding site of the probe. GBS=GBS1

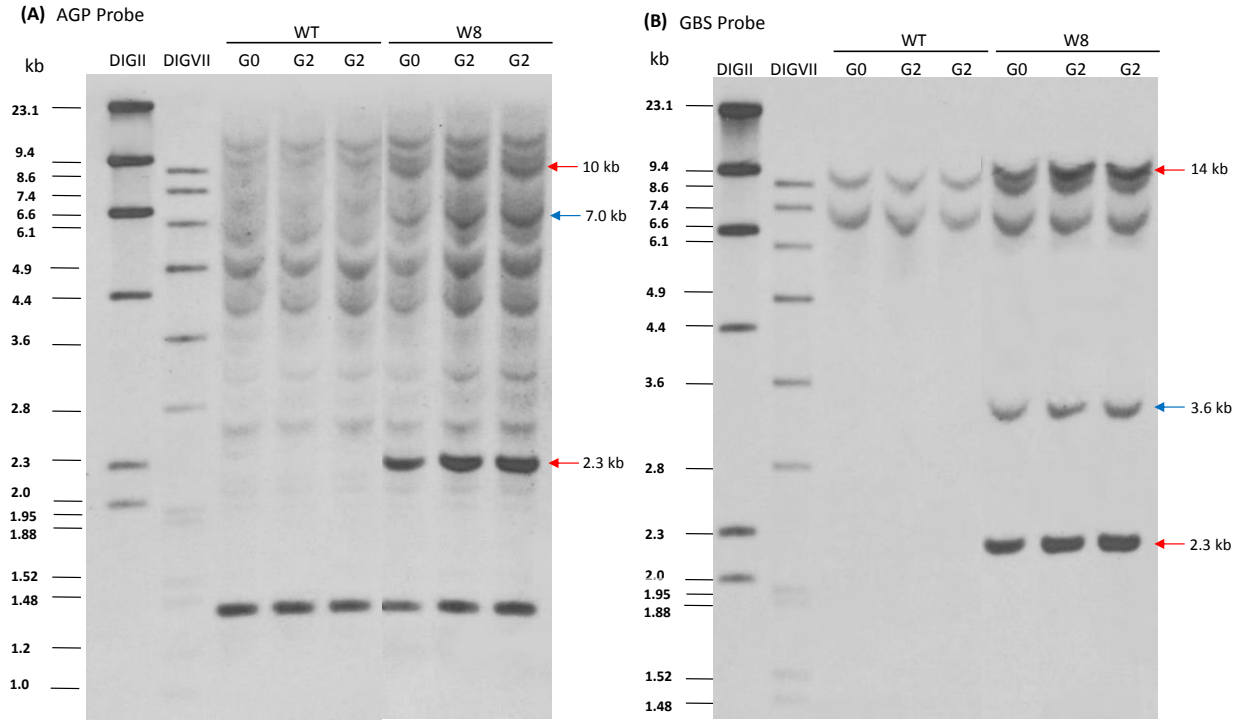


Figure 9. DNA Insert Stability in W8 with AGP and GBS Probes

Genomic DNA from G0 and G2 plants of Russet Burbank (WT) and W8 was digested by EcoRV and hybridised with the (A) AGP or (B) GBS probe. Red arrows indicate pSIM1278 insert bands and blue arrows indicate pSIM1678 insert bands. Molecular weight markers are DIGII and DIGVII, sizes are in kilobase pairs (kb). Note: genomic fragments may migrate faster than molecular weight markers due to the presence of residual polysaccharides in the isolated DNA. GBS=GBS1.

In addition, a second set of digests (XbaI) was analysed by Southern blot using the VNT and INV probes that are specific to the pSIM1678 insert. The 6.1 kb fragment covering the left junction of pSIM1678 was detected by the VNT probe in each of the W8 samples (Figure 10A). The INV probe detected the 4.6 and 5.5 kb fragments corresponding to the internal and right junction regions (compare Figure 8B with Figure 10B).

In all digests, the band size and intensity was uniform between G0 and G2 samples demonstrating stability of the inserts. Bands from endogenous potato genes were also observed and maintained through vegetative propagation.

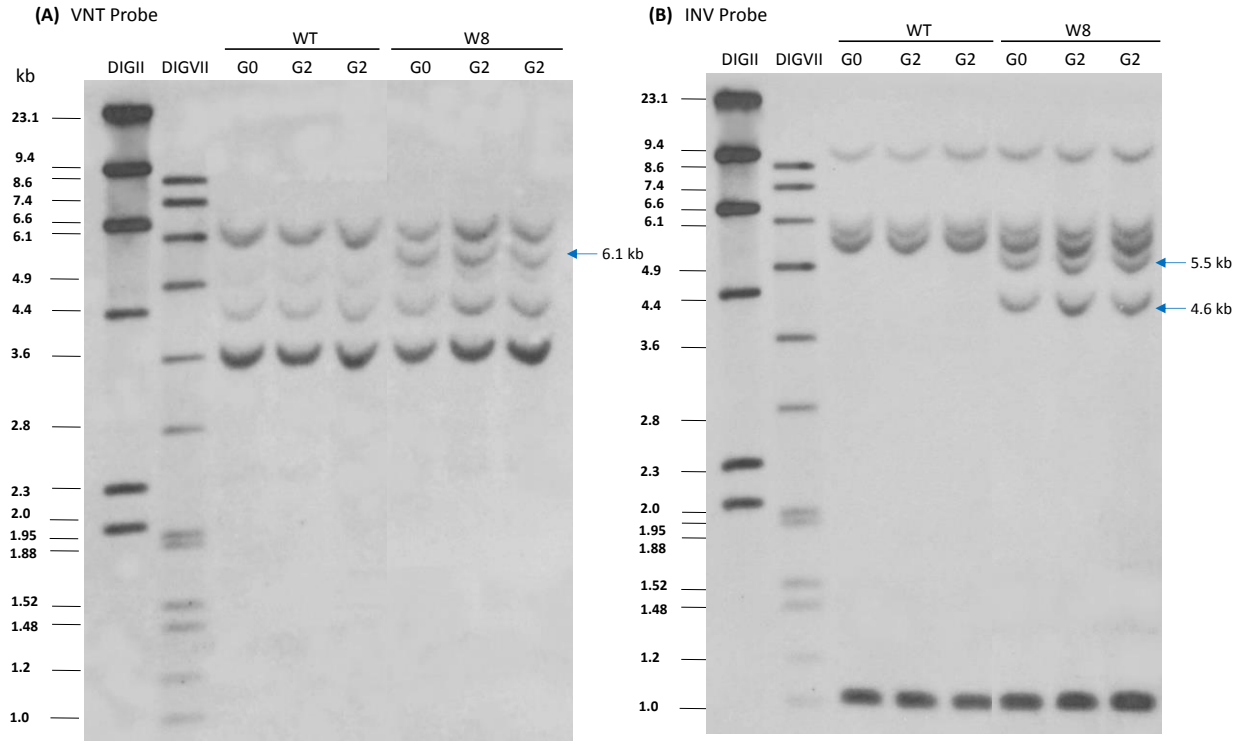


Figure 10. DNA Insert Stability in W8 with VNT and INV Probes

Genomic DNA isolated from G01 and G2 plants of Russet Burbank (WT) and W8 was digested by XbaI and hybridised with the (A) VNT or (B) INV probe. Blue arrows indicate pSIM1678 insert bands. Molecular weight markers are DIGII and DIGVII, sizes are in kilobase pairs (kb). Note: genomic fragments may migrate faster than molecular weight markers due to the presence of residual polysaccharides in the isolated DNA.

Conclusion for Event W8 Insert Stability

The Southern blot results showed consistent banding patterns with Russet Burbank and W8 plants, across two different time points. The consistent banding patterns demonstrated stability of both inserts in W8. As potatoes are vegetatively propagated, all progeny produced from an individual plant are genetically identical. Consequently, evaluating insert stability by examining inheritance using Mendelian segregation analysis is not applicable for potatoes. However, this study shows that the inserts in W8 are stable and maintained through vegetative propagation.

Stability of Event X17

Southern blot analyses verified the stability of the two DNA inserts in event X17 (Report 15-06-SPS-MOL). The stability of the X17 inserts were assessed in G0 and G2 tubers, demonstrating stability through vegetative propagation. X17 contains inserts from independent transformations with plasmids, pSIM1278 and pSIM1678. Six probes were used that hybridised to genetic elements in the inserts (Figure 11). The GBS1 and AGP probes hybridised to elements contained in both plasmids, whereas ASN and R1 probes were specific to pSIM1278 and VNT and INV are specific to pSIM1678. Consistent banding patterns on Southern blots provided evidence of genetic stability during vegetative propagation of X17. X17 and Ranger Russet DNA samples were digested with the restriction enzyme, EcoRV. The EcoRV restriction enzyme digests inserts from pSIM1278 and pSIM1678 into multiple bands that can be distinguished by size and probe specificity (Figure 11).

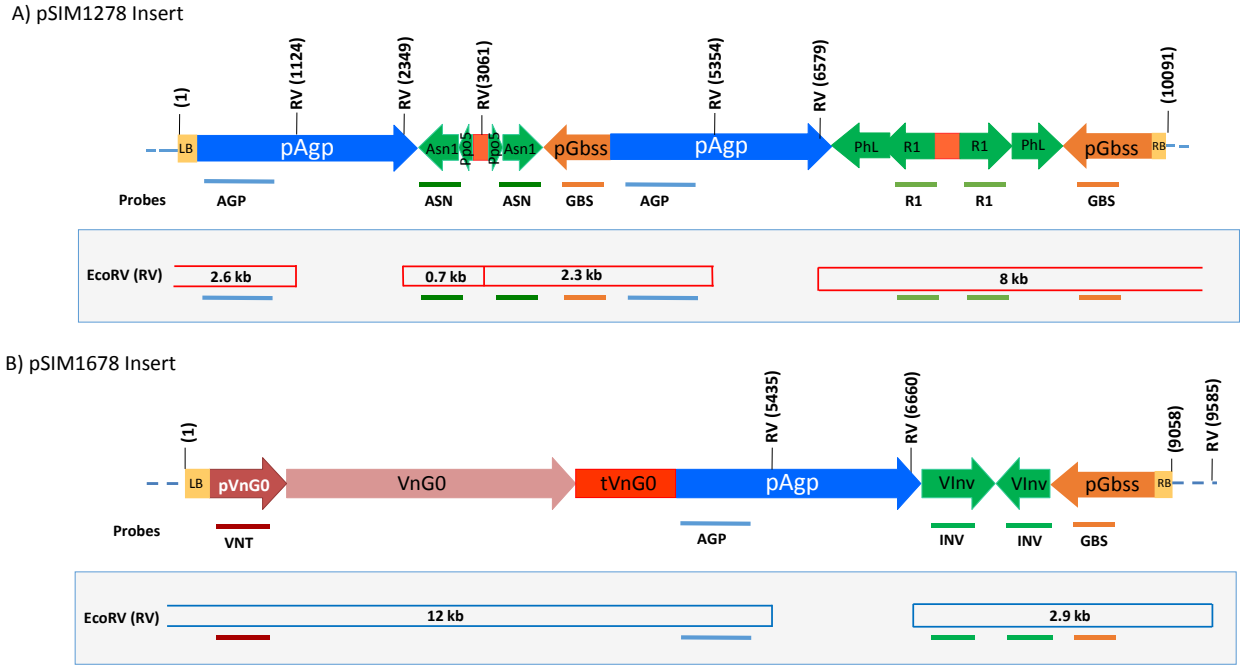


Figure 11. Structure of the pSIM1278 and pSIM1678 Inserts in X17

Structures of the (A) pSIM1278 insert and the (B) pSIM1678 insert in X17 are shown with EcoRV restriction sites located within the insert or in the nearby flanking region, where known. The bands expected from EcoRV digestion are shown below each image. Red boxes indicate bands associated with the pSIM1278 insert, while blue boxes indicate bands associated with the pSIM1678 insert. A coloured indicator below each box indicates the binding site for each probe. GBS = GBS1.

Red boxes (Figure 11) show that the EcoRV digest is expected to produce four bands (2.6 kb, 0.7 kb, 2.3 kb, and 8 kb) associated with the pSIM1278 insert. Southern blots hybridised separately with the AGP, GBS1, ASN, and R1 probes confirm the presence and size of these bands (Figure 12 and Figure 13). There were two bands (12 kb and 2.9 kb) associated with the EcoRV digest of the pSIM1678 insert (Figure 11).

Southern blots hybridised separately with the AGP, GBS1, INV, and VNT probes confirm the presence and size of these bands (Figure 12 and Figure 14).

The AGP and GBS probes hybridised to both inserts since the *Agp* and *Gbss* promoters are present in both inserts. The AGP probe hybridised to 2.3 kb and 2.6 kb bands associated with the pSIM1278 insert and an 11 kb band corresponding to the pSIM1678 insert (Figure 12A). Similarly, the GBS1 probe hybridised to 2.3 kb and 8 kb bands associated with the pSIM1278 insert and a 2.9 kb band associated with the pSIM1678 insert (Figure 12B). These bands were detected consistently in the X17 plants at different time points in vegetative production, indicating the stability of the inserts.

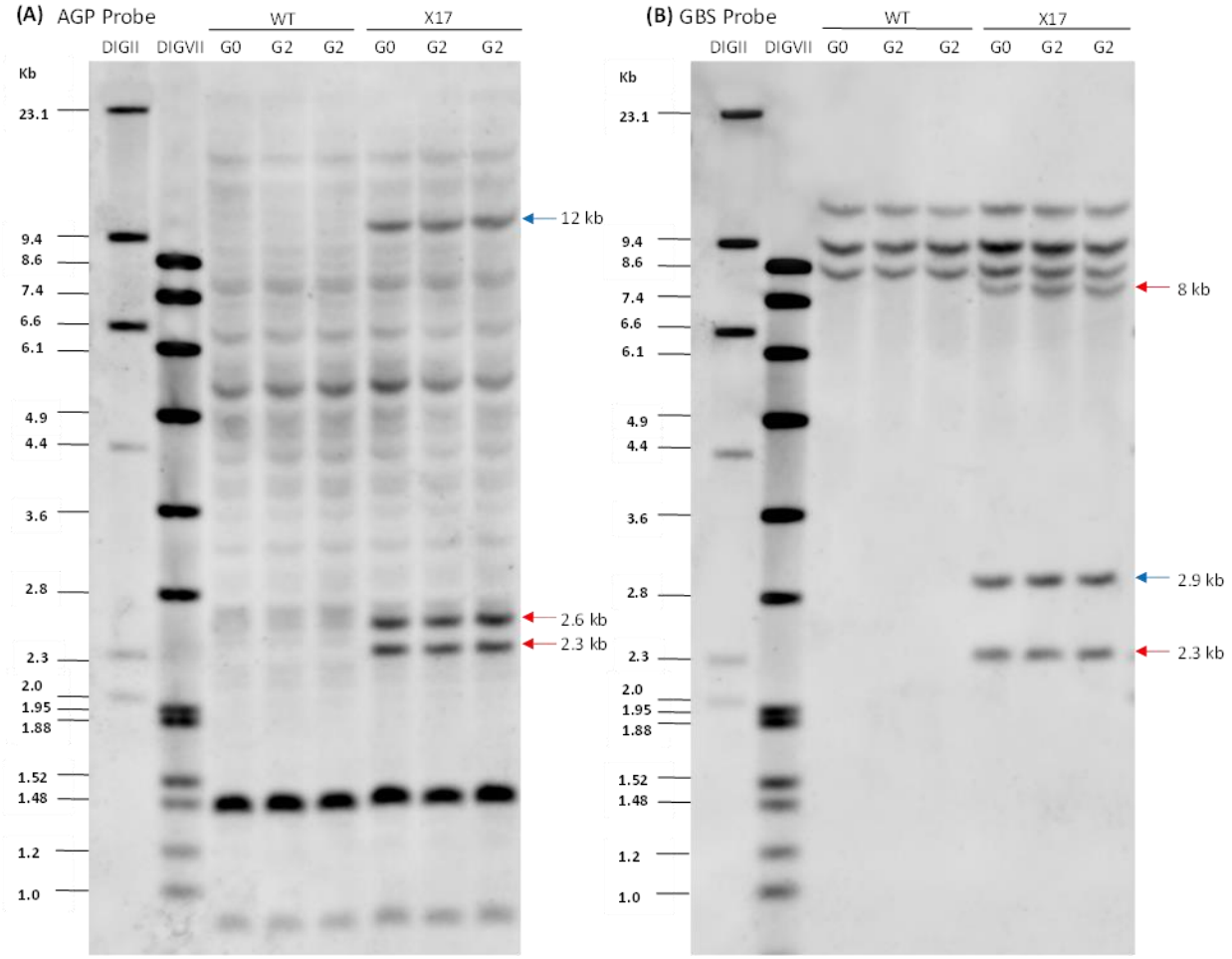


Figure 12. DNA Insert Stability in X17 Visualised with AGP and GBS Probes

Southern blots of genomic DNA isolated from G0 and G2 plants of Ranger Russet (WT) and X17 hybridised with (A) AGP probe or (B) GBS probe. GBS = GBS1. Red arrows indicate pSIM1278 insert bands and blue arrows indicate pSIM1678 insert bands. Molecular weight markers (DIGII and DIGVII) were included with band sizes indicated in kilobase pairs (kb).

The probes, ASN and R1, hybridised to genetic elements within the pSIM1278 insert but not the pSIM1678 insert. The ASN probe detected 0.7 kb and 2.3 kb bands Figure 13A), while the R1 probe detected the 8 kb band associated with the pSIM1278 insert (Figure 13B).

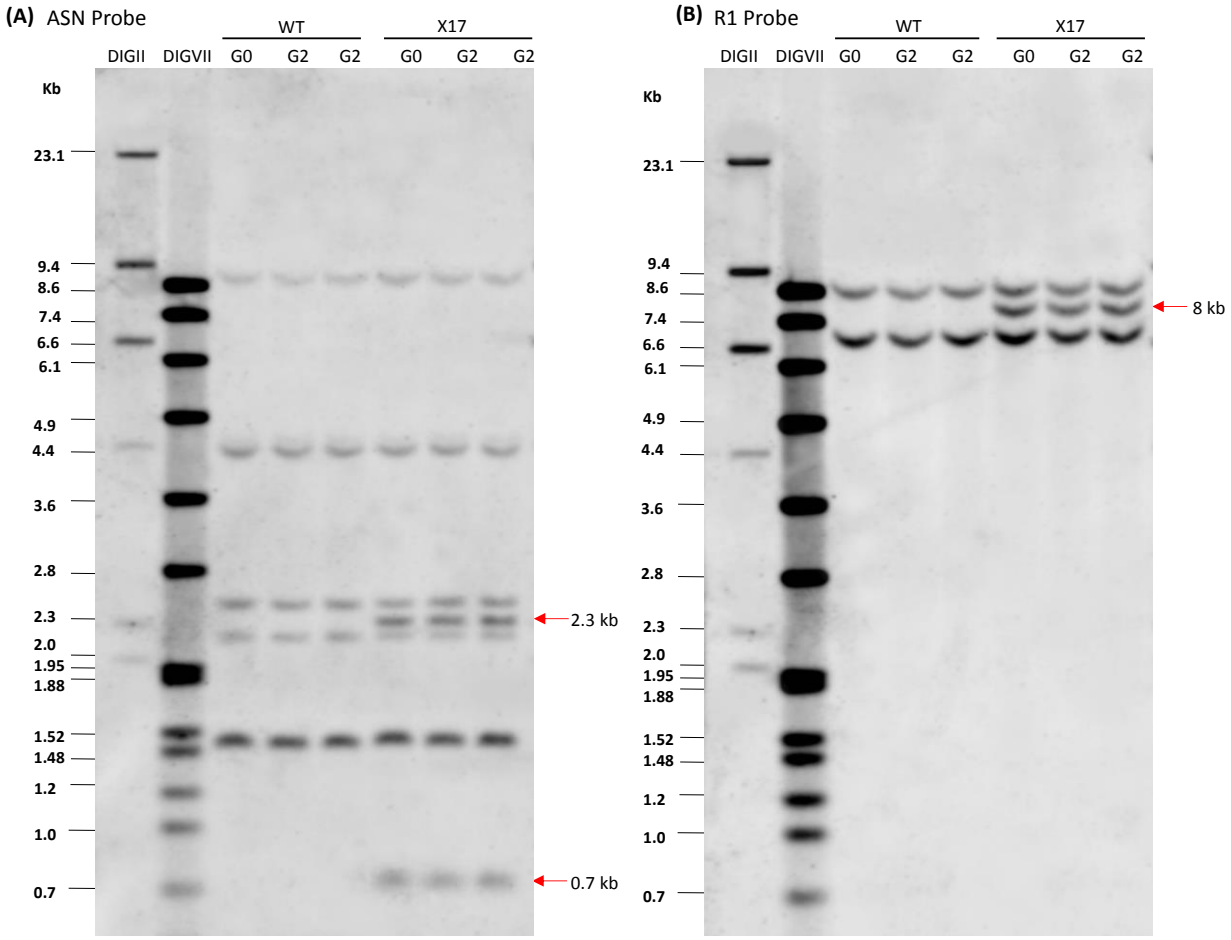


Figure 13. Insert Stability in X17 Visualised with ASN and R1 Probes

Southern blots of genomic DNA isolated from G0 and G2 plants of Ranger Russet (WT) and X17 hybridised with (A) ASN probe or (B) R1 probe. Red arrows indicate pSIM1278 insert bands. Molecular weight markers (DIGII and DIGVII) were included with band sizes indicated in kilobase pairs (kb).

The INV and VNT probes recognised opposite ends of the pSIM1678 insert (Figure 11). The INV probe hybridised to the 2.9 kb band while the VNT probe hybridised to the 12 kb band associated with the pSIM1678 insert (Figure 14). These bands were detected consistently in X17 plants from successive vegetative propagations, indicating the stability of the pSIM1678 insert. The banding patterns observed with Ranger Russet samples are from probe hybridisation to endogenous potato genes, which are also present in X17 (Figure 12, Figure 13, and Figure 14). These endogenous bands also remained constant between G0 and G2 samples.

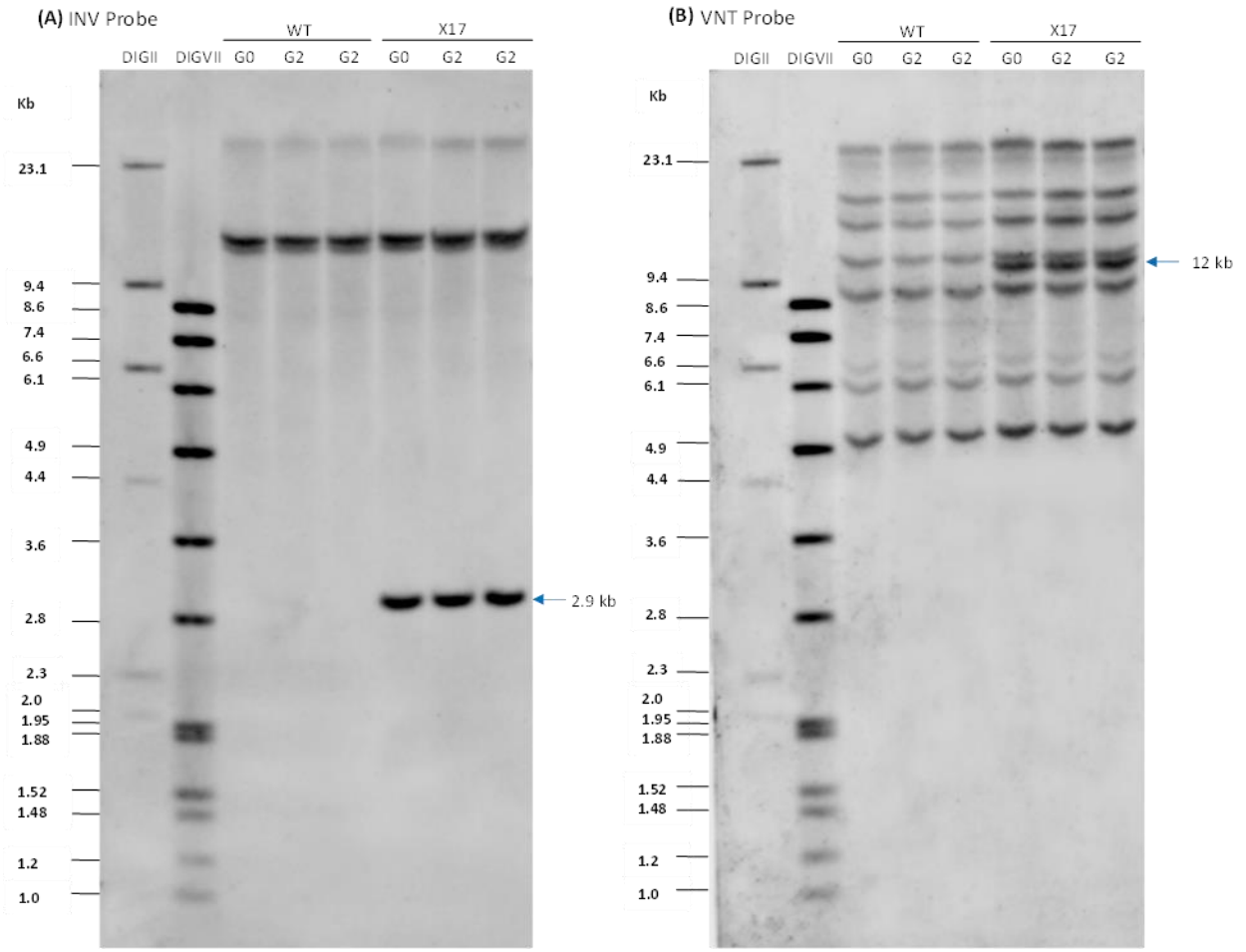


Figure 14. Insert Stability in X17 Visualised with INV and VNT Probes

Southern blots of genomic DNA isolated from G0 and G2 plants of Ranger Russet (WT) and X17 hybridised with (A) INV probe or (B) VNT probe. Blue arrows indicate pSIM1678 insert bands. Molecular weight markers (DIGII and DIGVII) were included with band sizes indicated in kilobase pairs (kb).

<u>Conclusion for Event X17 Insert Stability</u>

The Southern blot results showed consistent banding patterns between G0 and G2 vegetative propagations for X17. The banding patterns indicated no changes in the structure of the two inserts in X17. As potatoes are vegetatively propagated, all progeny produced from an individual plant are genetically identical. Consequently, evaluating insert stability by examining inheritance using Mendelian segregation analysis is not applicable for potatoes. This study shows that the inserts in X17 are stable and maintained through vegetative propagation.

Stability of Event Y9

Southern blot analyses were conducted to verify the stability of the two DNA inserts in event Y9 (Report 15-14-SPS-MOL). As Y9 contains inserts from independent transformations with plasmids pSIM1278 and pSIM1678, four probes were used that hybridise to genetic elements in each insert (Figure 15). Consistent banding patterns on Southern blots provide evidence of genetic stability among Y9 progeny between G0 and G2 samples. Y9 and Atlantic (WT) DNA were digested with the restriction enzyme, EcoRV. The EcoRV restriction enzyme digests both the pSIM1278 and pSIM1678 insert into multiple bands that can be distinguished by size and probe specificity (Figure 15).

The red boxes in Figure 15 show that the EcoRV digest is expected to produce six fragments (7 kb, 0.7 kb, 1.6 kb, 0.7 kb, 2.3 kb, and 16 kb) associated with the pSIM1278 insert that are detected by probes: AGP, GBS, ASN, or R1. Southern blots hybridised separately with the probes confirmed the presence and size of these bands (Figure 16 and Figure 17). There were two bands (7.4 kb and 5.1 kb) from the EcoRV digest associated with the pSIM1678 insert that are detected by probes: VNT, AGP, INV, and GBS (blue boxes; Figure 15). Southern blots hybridised separately with these probes confirmed the presence and size of these bands (Figure 16 and Figure 18).

The AGP and GBS probes hybridise to both inserts since the *Agp* and *Gbss* promoters are present in both inserts. The AGP probe hybridised to three bands (7 kb, 1.6 kb, and 2.3 kb) associated with the pSIM1278 insert and the 7.4 kb band corresponding to the pSIM1678 insert (Figure 16A). Similarly, the GBS probe hybridised to three bands (7 kb, 2.3 kb, and 16 kb) associated with the pSIM1278 insert and the 5.1 kb band associated with the pSIM1678 insert (Figure 16B). These bands were detected consistently in the Y9 plants at different time points in vegetative production, indicating insert stability.

The probes, ASN and R1, hybridised to genetic elements within the pSIM1278 insert but not the pSIM1678 insert. The ASN probe detected three bands (7 kb, 0.7 kb, 2.3 kb; Figure 17A), while the R1 probe detected the 16 kb band associated with the pSIM1278 insert (Figure 17B). The INV and VNT probes recognise opposite ends of the pSIM1678 insert (Figure 15). The INV probe hybridised to the 5.1 kb band while the VNT probe hybridised to the 7.4 kb band associated with the pSIM1678 insert (Figure 18). These bands were detected consistently in the Y9 plants through vegetative propagation indicating pSIM1678 insert stability. The banding patterns of Atlantic samples are from probes hybridising to endogenous potato genes, which are also present in Y9 (Figure 16, Figure 17, and Figure 18). These endogenous bands remained constant between G0 and G2 samples.

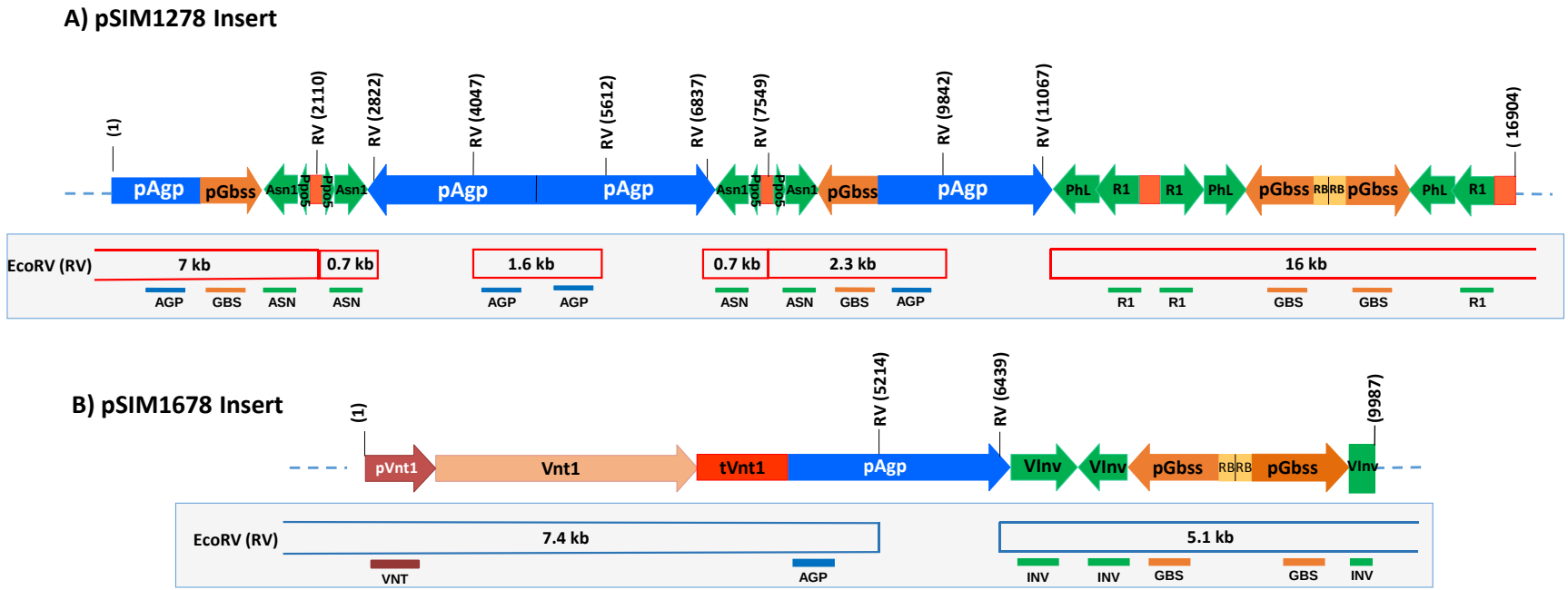


Figure 15. Structure of the pSIM1278 and pSIM1678 Insert in Y9

Structure of the (A) pSIM1278 insert and the (B) pSIM1678 insert in Y9 with the EcoRV restriction sites located within the insert or in the nearby flanking region, where known. The bands expected from EcoRV digestion are shown below each image. Red boxes indicate bands associated with the pSIM1278 insert, while blue boxes indicate bands associated with the pSIM1678 insert. A coloured indicator below each box indicates the binding site for each probe.

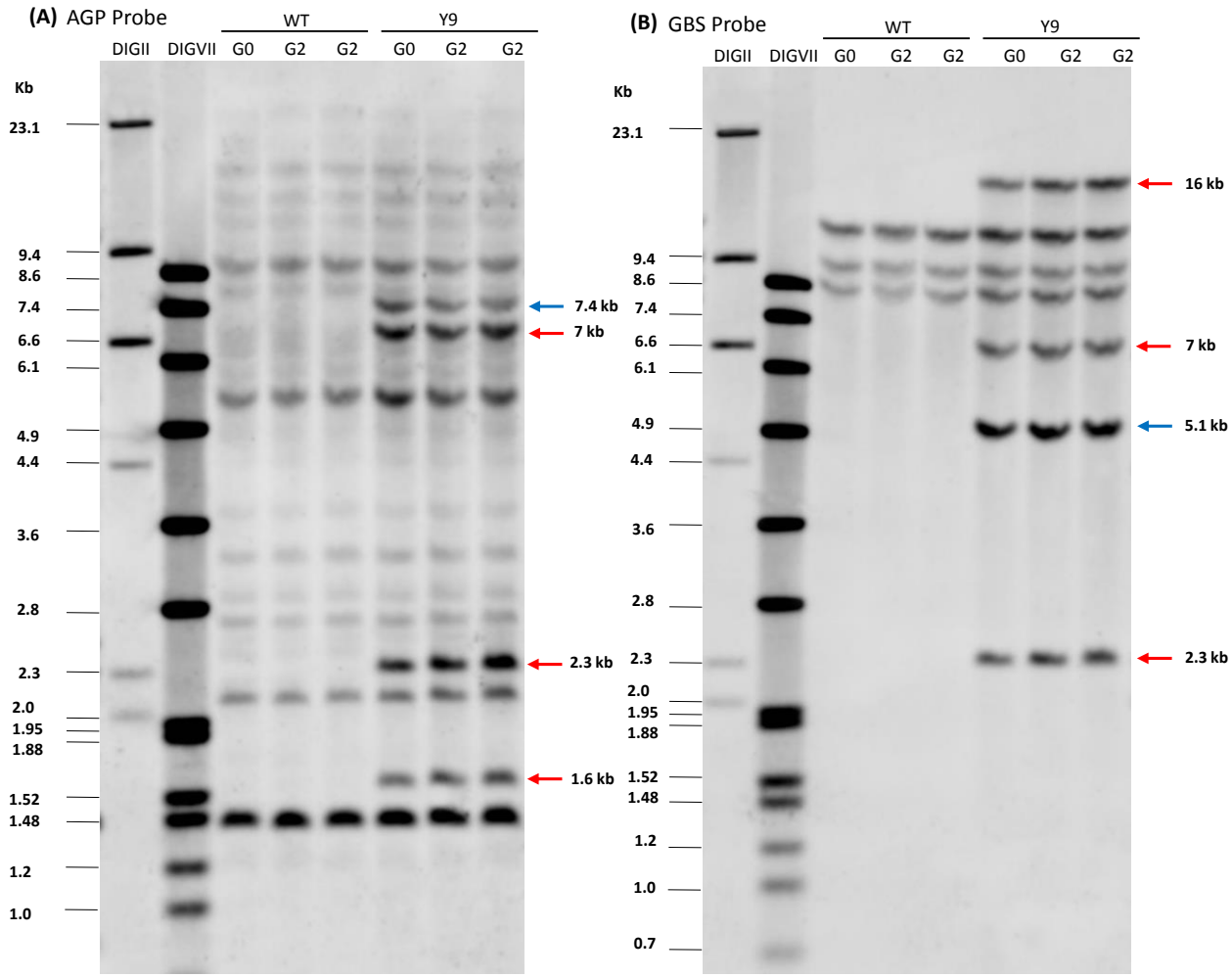


Figure 16. Insert Stability in Y9 Visualised with AGP and GBS Probes

Southern blots of genomic DNA isolated from G0 and G2 plants of Atlantic (WT) and Y9 hybridised with (A) AGP probe or (B) GBS probe. Red arrows indicate pSIM1278 insert bands and blue arrows indicate pSIM1678 insert bands. Molecular weight markers (DIGII and DIGVII) were included with band sizes indicated in kilobase pairs (kb).

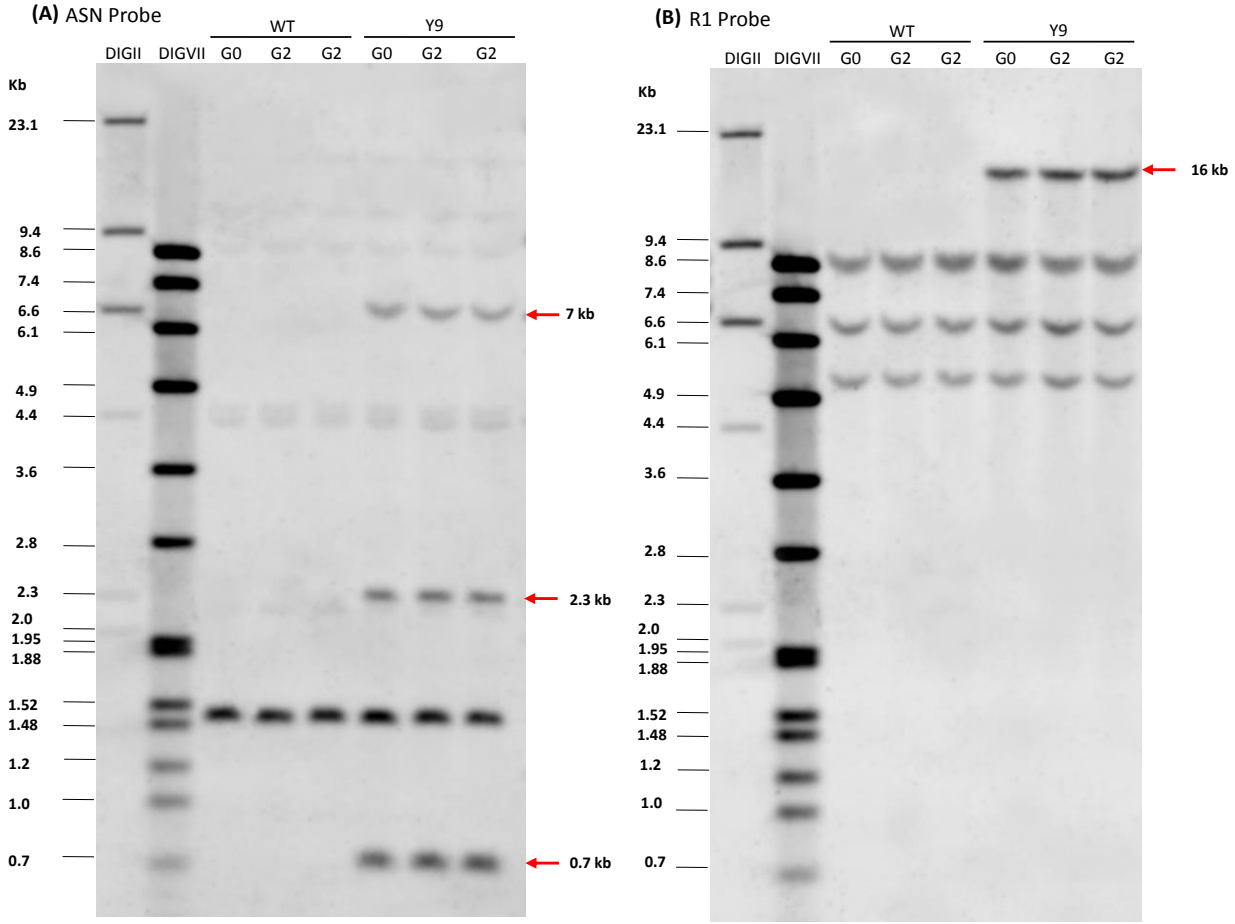


Figure 17. Insert Stability in Y9 Visualised with ASN and R1 Probes

Southern blots of genomic DNA isolated from G0 and G2 plants of Atlantic (WT) and Y9 hybridised with (A) ASN probe or (B) R1 probe. Red arrows indicate pSIM1278 insert bands. Molecular weight markers (DIGII and DIGVII) were included with band sizes indicated in kilobase pairs (kb).

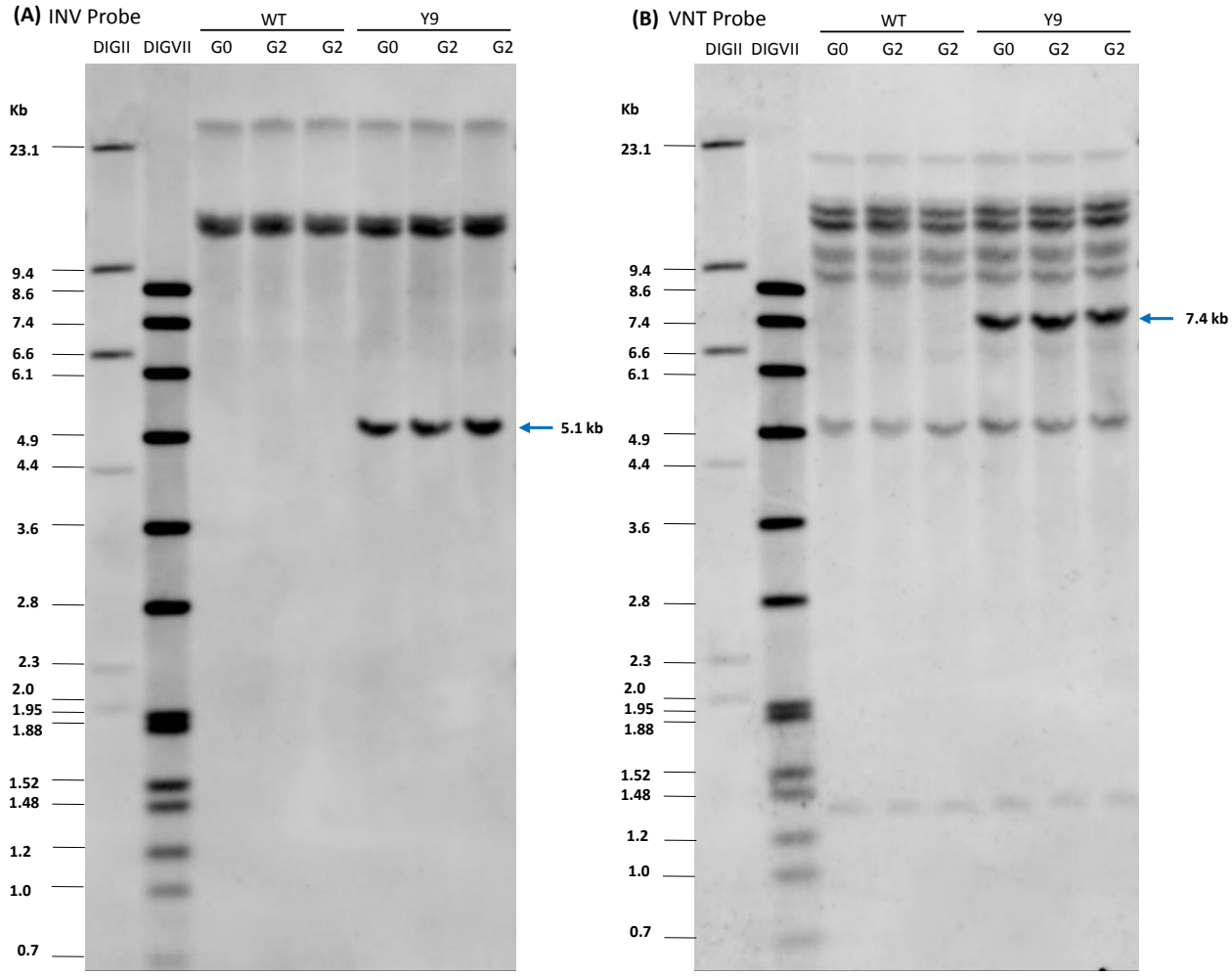


Figure 18. Insert Stability in Y9 Visualised with INV and VNT Probes

Southern blots of genomic DNA isolated from G0 and G2 plants of Atlantic (WT) and Y9 hybridised with (A) INV probe or (B) VNT probe. Blue arrows indicate pSIM1678 insert bands. Molecular weight markers (DIGII and DIGVII) were included with band sizes indicated in kilobase pairs (kb).

Conclusion for Event Y9 Insert Stability

The Southern blot results showed consistent banding patterns across G0 and G2 vegetative propagations for Y9. The banding patterns indicated no changes in the insert structure of the two inserts in Y9. As potatoes are vegetatively propagated, all progeny produced from an individual plant are genetically identical. Consequently, evaluating insert stability by examining inheritance using Mendelian segregation analysis is not applicable for potatoes. This study shows that the inserts in Y9 are stable and maintained through vegetative propagation.

Summary of Genetic Stability Studies

Southern blot testing showed consistent banding patterns for W8, X17, and Y9 across three cycles of vegetative propagation, demonstrating genetic stability. The stability of the pSIM1278 insert in W8, X17, and Y9 demonstrated that the pSIM1278 insert remained unchanged following transformation with pSIM1678. It is expected that stability of the inserts will be maintained with vegetative propagation.

A.3(g) An analysis of the expressed RNA transcripts, where RNA interference has been used

In W8, X17, and Y9, reduced black spot, reduced free asparagine, and lower reducing sugars were achieved using RNAi to target five potato RNA transcripts for down regulation (Table 2).

The promoters that drive down regulation are primarily active in tubers. The effectiveness of target gene down regulation and tissue specificity was evaluated by comparing the mRNA levels of the targeted transcripts in tubers, leaves, stems, roots, and flowers in each event using northern blot analysis.

Black spot is a post-harvest physiological phenomenon primarily resulting from the handling of potato tubers during harvest, transport, and processing, and refers to the black or greyish colour that may form in the interior of damaged potatoes. The enzymatic darkening and discolouration, associated with the enzyme polyphenol oxidase (PPO), occurs when PPO leaks out from the plastids of damaged potatoes. Potatoes that show black spot are typically trimmed, or oftentimes the entire potato is rejected before processing. This results in quality control challenges, economic loss, or both. The PPO cassette in pSIM1278 targets polyphenol oxidase transcripts to down regulate enzyme expression via RNAi.

Target Transcript Down Regulation in W8

The results show reduced expression for four of the five target transcripts in tubers (water dikinase was not down regulated in tubers). Except for a small reduction in asparagine synthetase levels in flowers, expression of the five targeted transcripts in other tissues was unaffected. The 18S rRNA levels remained consistent across samples allowing for direct comparisons of asparagine synthetase, polyphenol oxidase, phosphorylase L and water dikinase transcripts between samples in a given tissue. Results are summarised in Table 7. Three biological replicates of W8 and Russet Burbank (WT) were evaluated in each gel.

Table 7. Summary of Down-Regulated Transcripts in W8 Plants

Transcript	Tuber	Leaf	Stem	Root	Flower
Asparagine synthetase	✓	-	-	-	✓
Polyphenol oxidase	✓	-	-	-	-
Phosphorylase L	✓	-	-	-	-
Water dikinase	-	-	-	-	-
Vacuolar invertase	✓	-	-	-	-

✓ = down regulated, - = not down regulated.

W8 Tuber

The absence or lower intensity of bands in the W8 samples indicated down regulation of transcripts in W8 tubers compared to the Russet Burbank (WT) (Figure 19). The down regulation was particularly strong as nearly complete absence of the target RNA signal was observed. Down regulation was not observed for tuber samples probed for water dikinase transcripts.

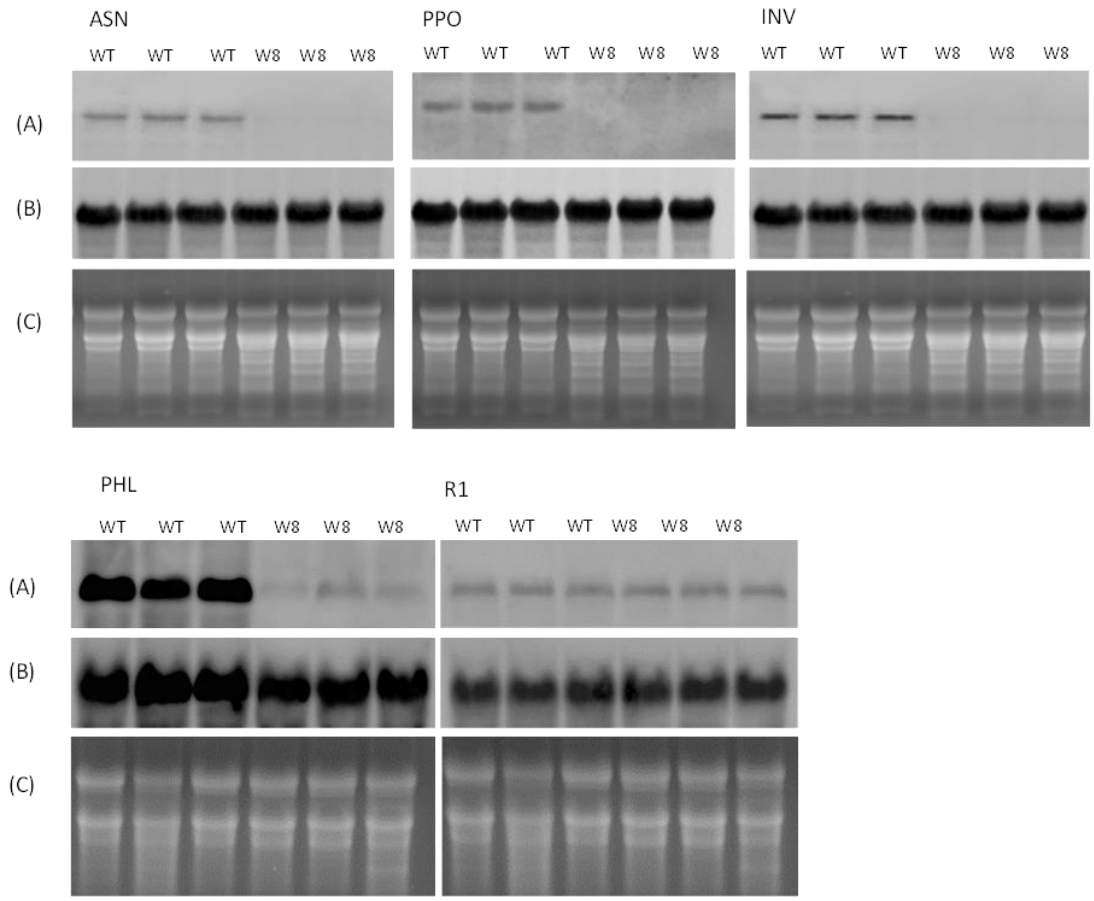


Figure 19. Target Transcript Down Regulation in W8 Tubers

Northern blots hybridised with the indicated probes to detect targeted transcripts. (A) Tuber samples analysed with the indicated probes. (B) 18S rRNA gel loading control. (C) Total RNA stained with ethidium bromide.

W8 Leaf

Down regulation was not observed for any of the transcripts in W8 leaf samples compared to the Russet Burbank (WT), consistent with down regulation specific to tubers (Figure 20).

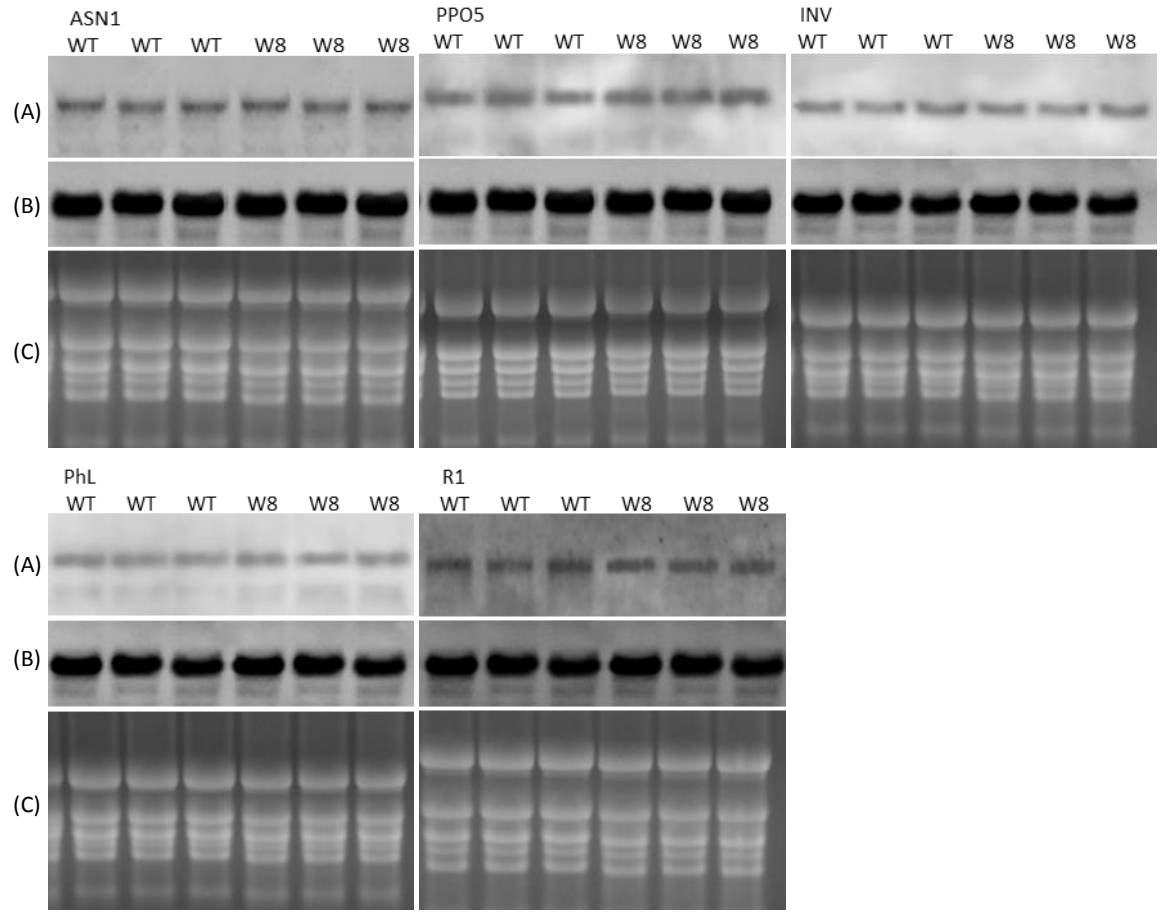


Figure 20. No Changes in Target Transcript Expression in W8 Leaves

Northern blots hybridised with the indicated probes to detect targeted transcripts. (A) Leaf samples analysed with the indicated probes. (B) 18S rRNA gel loading control. (C) Total RNA stained with ethidium bromide.

W8 Stem

Down regulation was not observed for any of the transcripts in W8 stem samples compared to the Russet Burbank (WT), consistent with down regulation specific to tubers (Figure 21).

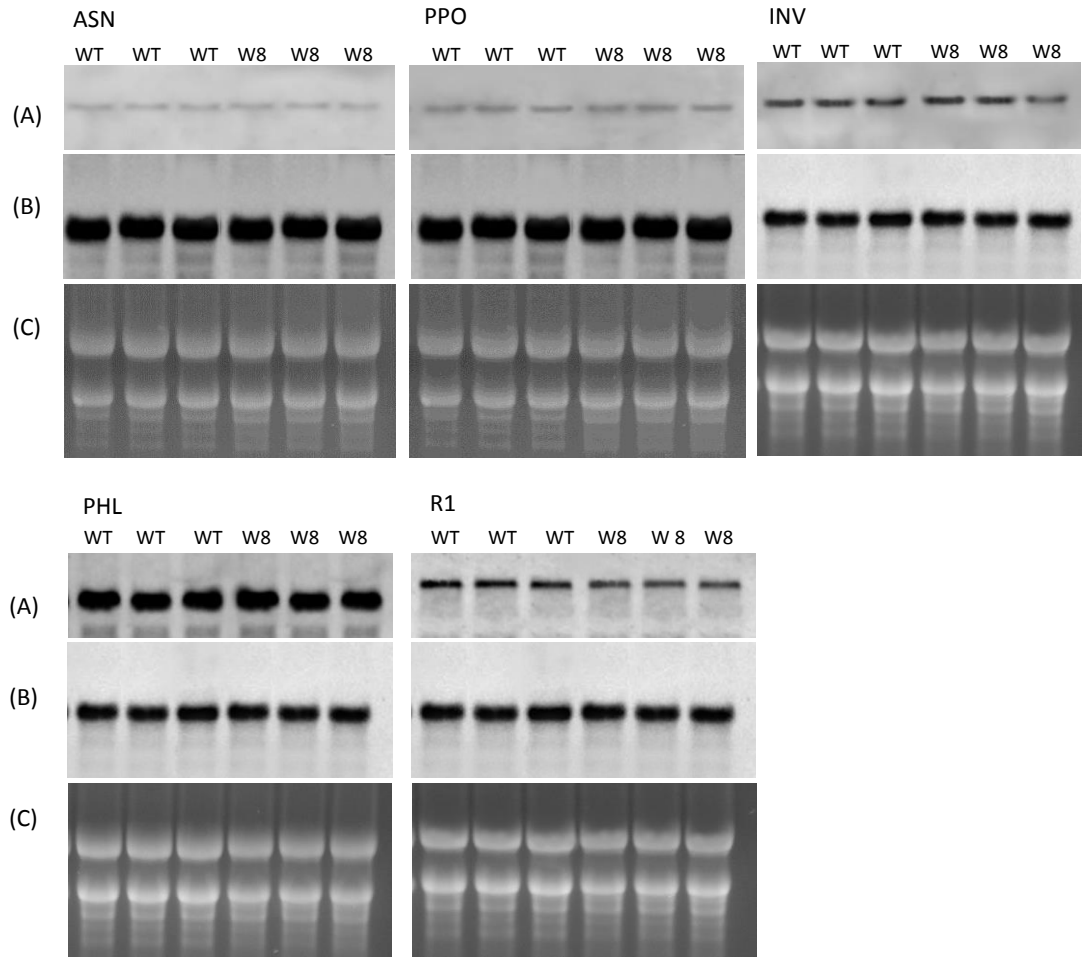


Figure 21. No Changes in Target Transcript Expression in W8 Stems

Northern blots hybridised with the indicated probes to detect targeted transcripts. (A) Stem samples analysed with the indicated probes. (B) 18S rRNA gel loading control. (C) Total RNA stained with ethidium bromide.

W8 Root

Down regulation was not observed for any of the transcripts in W8 root samples compared to the Russet Burbank (WT), consistent with down regulation specific to tubers (Figure 22).

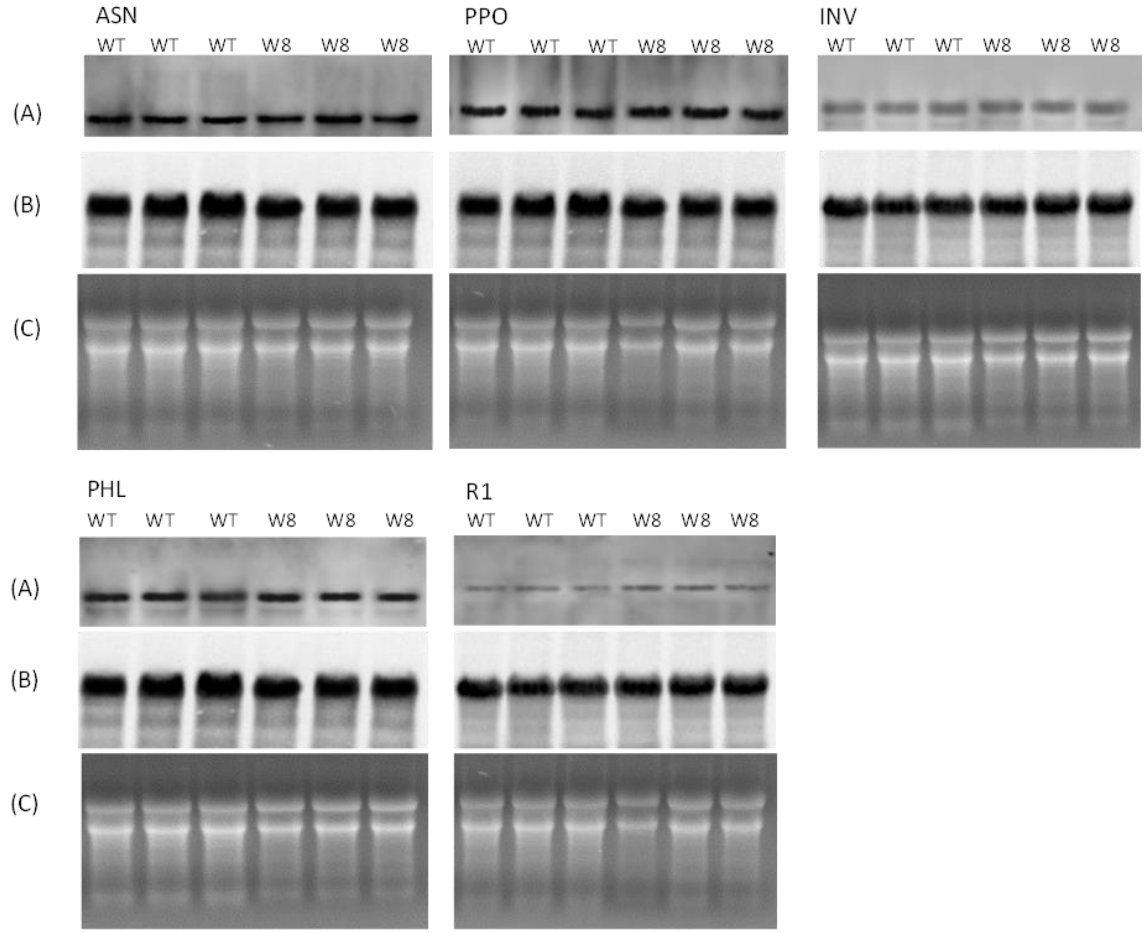


Figure 22. No Changes in Target Transcript Expression in W8 Roots

Northern blots hybridised with probes to detect targeted transcripts. (A) Root samples analysed with the indicated probes. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

W8 Flower

A partial decrease in asparagine synthetase transcript expression was observed in W8 flower samples (Figure 23). There was no observable down regulation of other transcripts in W8 flower samples consistent with down regulation specific to tubers (Figure 23).

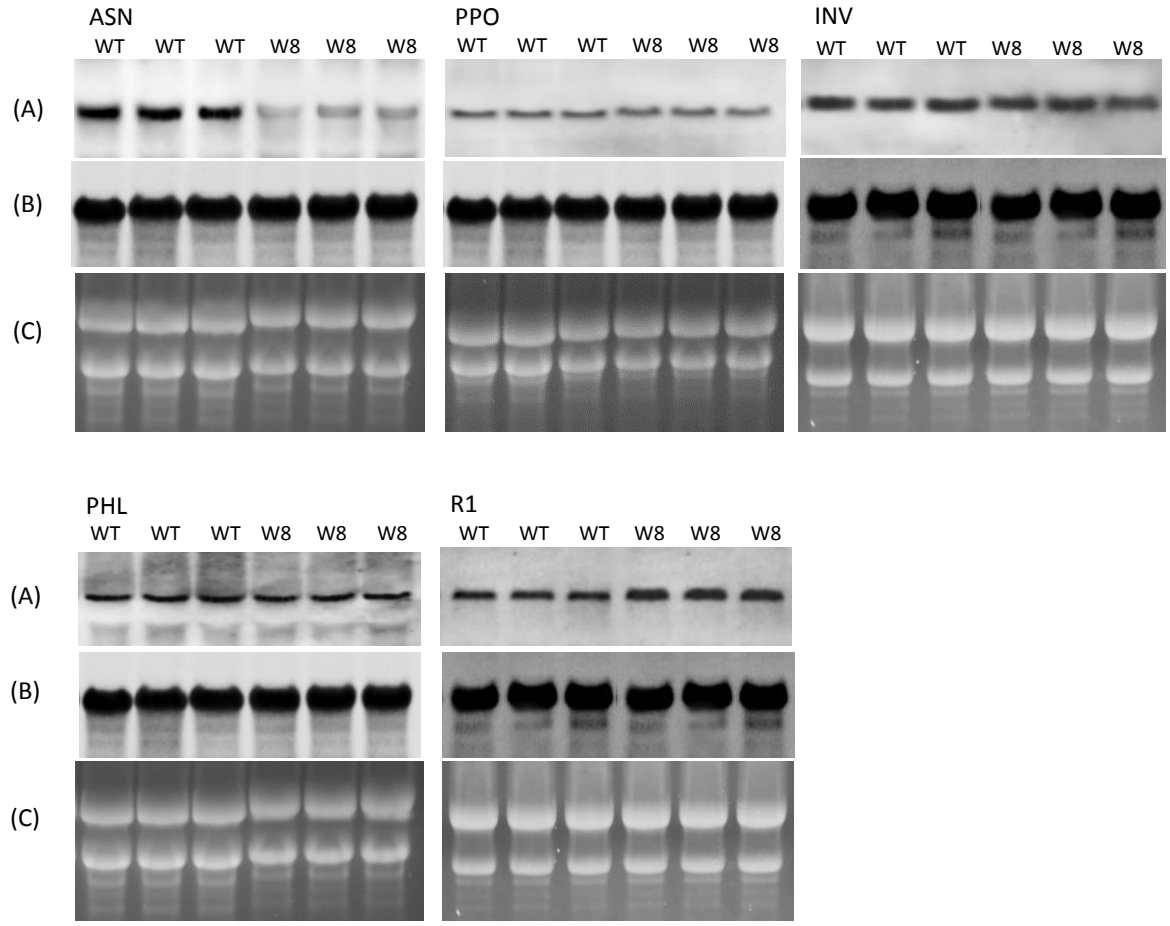


Figure 23. Target Transcript Down Regulation in W8 Flowers

Northern blots hybridised with the indicated probes to detect targeted transcripts. (A) Flower samples analysed with the indicated probes. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

Summary of Target Transcript Down Regulation in W8

The northern blot results showed reduced expression for four of the five target transcripts in W8 tubers (water dikinase was not down regulated in tubers). Except for a small reduction in asparagine synthetase mRNA levels in flowers, expression of the five targeted transcripts in leaf, stem, root and flower was unaffected in W8.

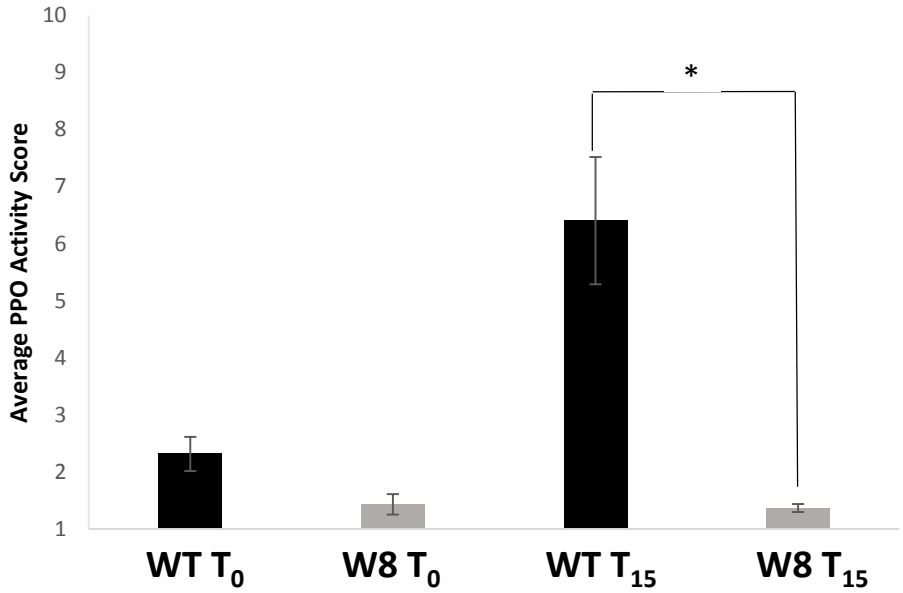
PPO Activity in Event W8

The trait efficacy of polyphenol oxidase (PPO) down regulation in potato event W8 was compared with Russet Burbank (Report15-85-SPS-MOL). A field trial was conducted in Canyon County, Idaho during the 2015 growing season. Plots of W8 and Russet Burbank were harvested, and tubers were assessed for the darkening associated with the PPO enzyme using a colorimetric assay.

PPO activity was measured by monitoring the conversion of an exogenous diphenolic substrate, catechol, into melanin which is associated with a visible colour change. An exogenous substrate was used to increase the rate and extent of colour change in the samples.

Ten tubers from field grown W8 and Russet Burbank were analysed using the PPO activity assay in triplicate.

The colour change associated with each sample was scored using the PPO Activity Colorimetric Scoring Scale with a summary of the results depicted in Figure 24. After 15 minutes, the colour of W8 samples was similar to the T_0 time point, whereas significant colour change occurred in the WT samples after 15 minutes (Figure 24). The difference between the WT and W8 T_{15} samples was statistically significant ($p<0.01$). These results confirm a reduction of PPO activity in W8 tubers.



	WT T_0	W8 T_0	WT T_{15}	W8 T_{15}
Mean	2.317	1.433	6.400	1.367
Std Dev	0.299	0.179	1.114	0.070

Figure 24. Reduced PPO Activity in W8 Compared with Russet Burbank

PPO activity was scored using the PPO Activity Colorimetric Scoring Scale for each of the 10 biological samples. The mean and standard deviation (Std Dev) values are listed above (n=10). The data compares activity scores between W8 and Russet Burbank (WT) at T_0 and T_{15} in the presence of catechol. The average PPO activity score at 15 minutes catechol exposure is significantly lower in W8 when compared with Russet Burbank (* denotes $p<0.01$).

Target Transcript Down Regulation in X17

The northern blot results showed that asparagine synthetase, polyphenol oxidase and vacuolar invertase transcripts were down regulated in tubers (Figure 25). Phosphorylase L and water dikinase transcripts showed no down regulation. Partial down regulation of asparagine synthetase in leaf and asparagine synthetase and vacuolar invertase transcripts in flower was observed. The 18S rRNA levels remained consistent across samples allowing for direct comparisons of transcripts between samples in a given tissue. Results are summarised in Table 8. Three biological replicates of X17 and Ranger Russet (WT) were evaluated in each gel.

Table 8. Summary of Targeted Down-Regulated Transcripts in X17 Plants

Transcript	Tuber	Leaf	Stem	Root	Flower
Asparagine synthetase	✓	✓	-	-	✓
Polyphenol oxidase	✓	-	-	-	-
Phosphorylase L	-	-	-	-	-
Water dikinase	-	-	-	-	-
Vacuolar invertase	✓	-	-	-	✓

✓ = down regulated, - = not down regulated.

X17 Tuber

The absence or decreased intensity of bands in the X17 samples probed for asparagine synthetase, polyphenol oxidase and vacuolar invertase transcripts indicated down regulation in X17 tubers compared to Ranger Russet (WT) (Figure 25). The extent of down regulation varied, with polyphenol oxidase and vacuolar invertase transcript down regulation being particularly strong, showing nearly complete absence of the transcript RNA signal. Decreased transcript expression was observed for the asparagine synthetase, while no decrease was observed for phosphorylase L and water dikinase in X17 tubers.

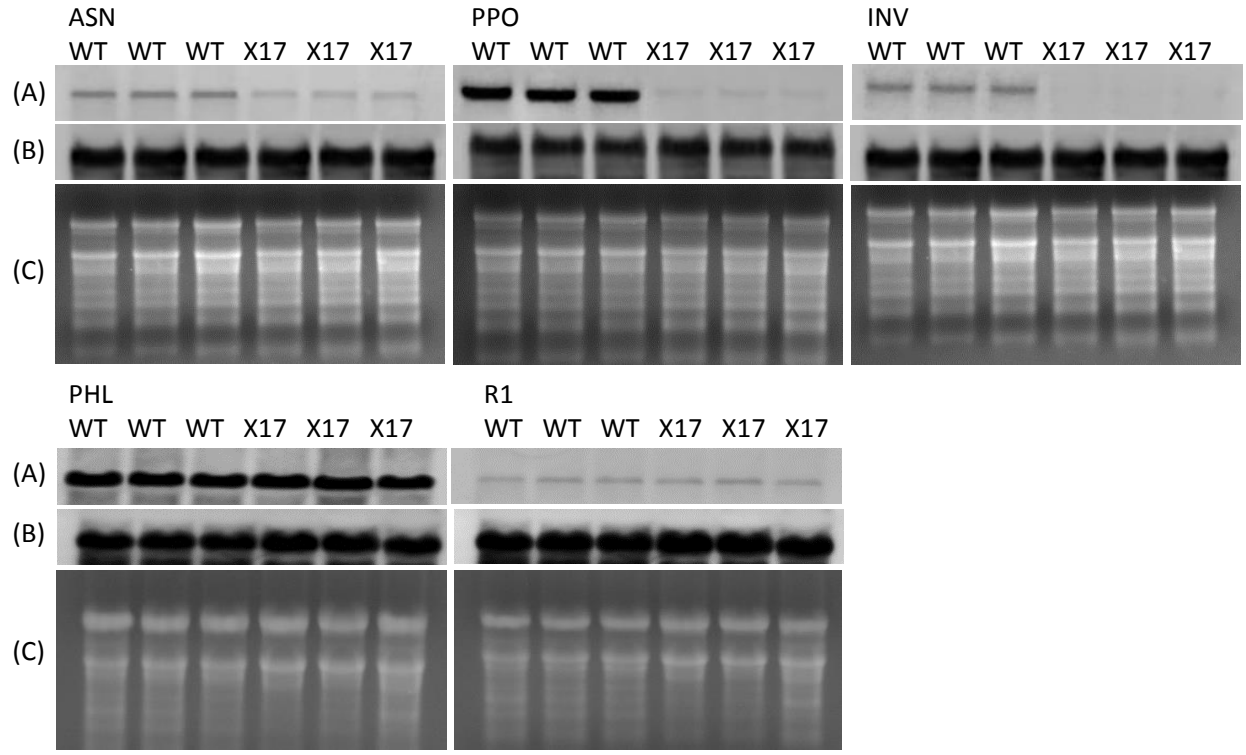


Figure 25. Target Transcript Down Regulation in X17 Tubers

(A) Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

X17 Leaf

A partial decrease in asparagine synthetase transcripts was observed in X17 leaf samples (Figure 26). There was no observable down regulation of the other transcripts in X17 leaf samples compared to Ranger Russet (WT), consistent with tuber specific down regulation (Figure 26).

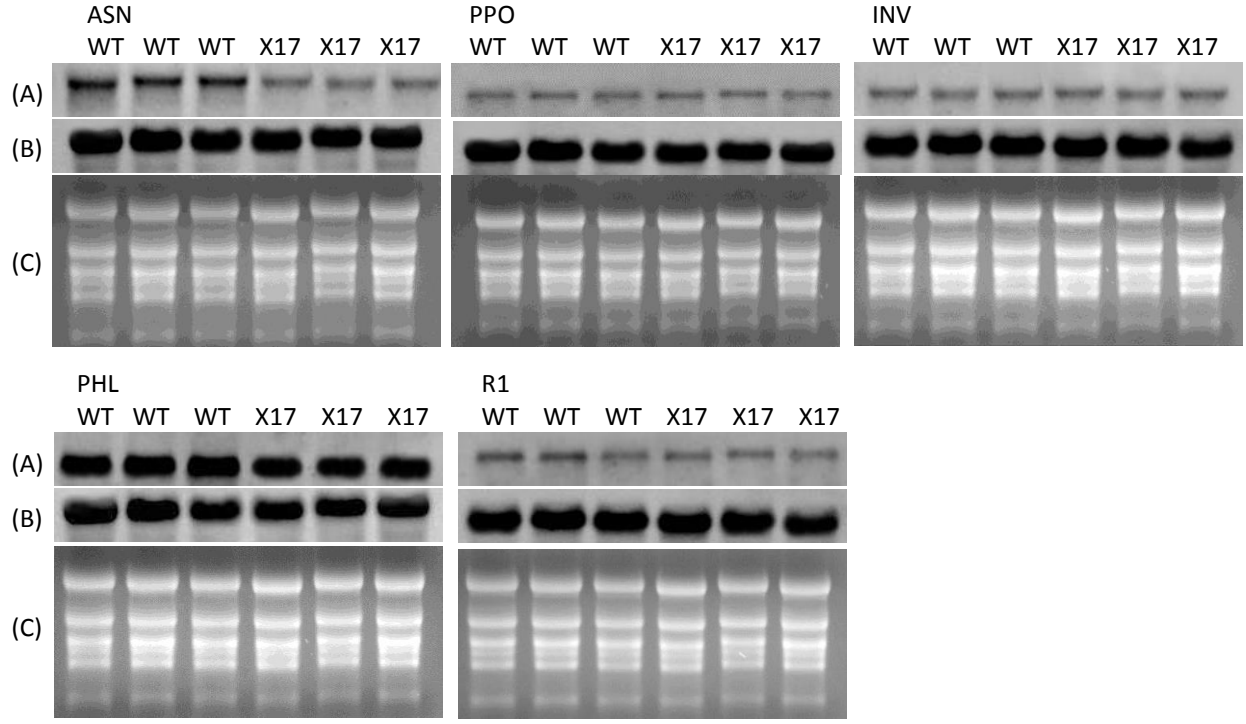


Figure 26. Minor Changes in Asparagine Synthetase Transcript Expression in X17 leaves

(A) Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

X17 Stem

Down regulation was not observed for any of the target transcripts in X17 stem samples compared to Ranger Russet (WT), consistent with down regulation specific to tubers (Figure 27).

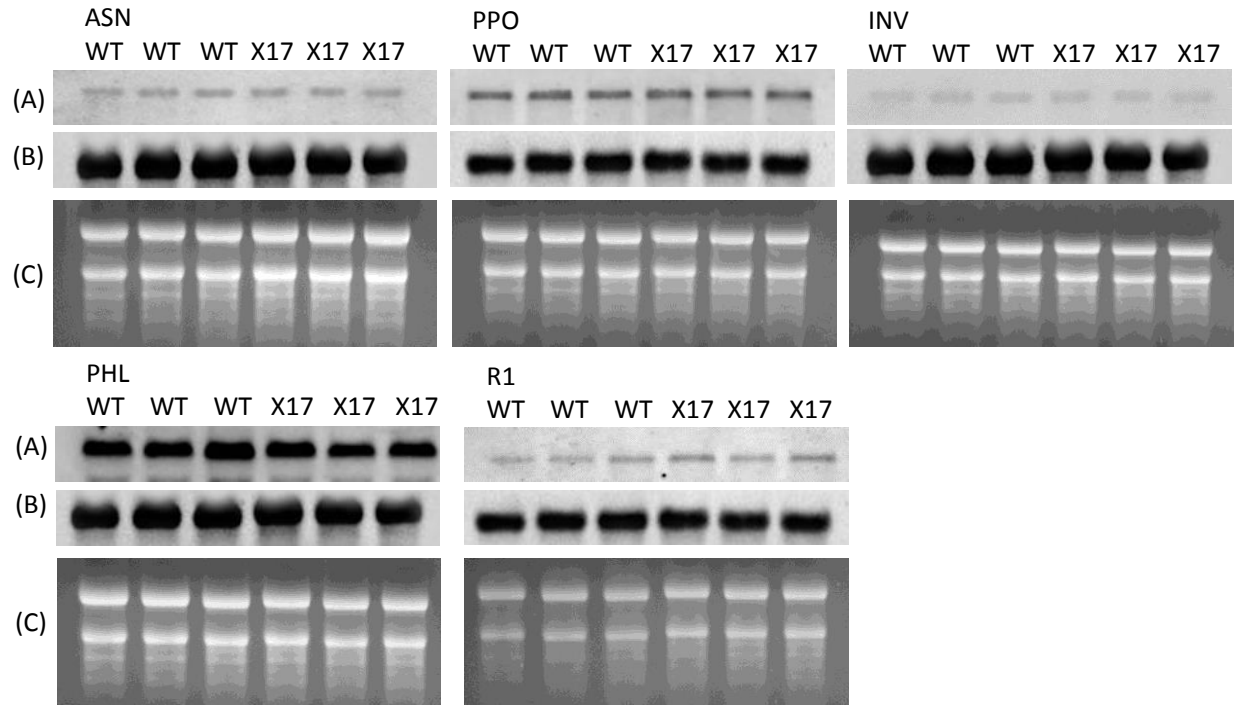


Figure 27. No Effect on Target Transcript Expression in X17 Stems

(A) Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

X17 Root

Down regulation was not observed for any target transcripts in X17 root samples compared to Ranger Russet (WT), consistent with down regulation specific to tubers (Figure 28).

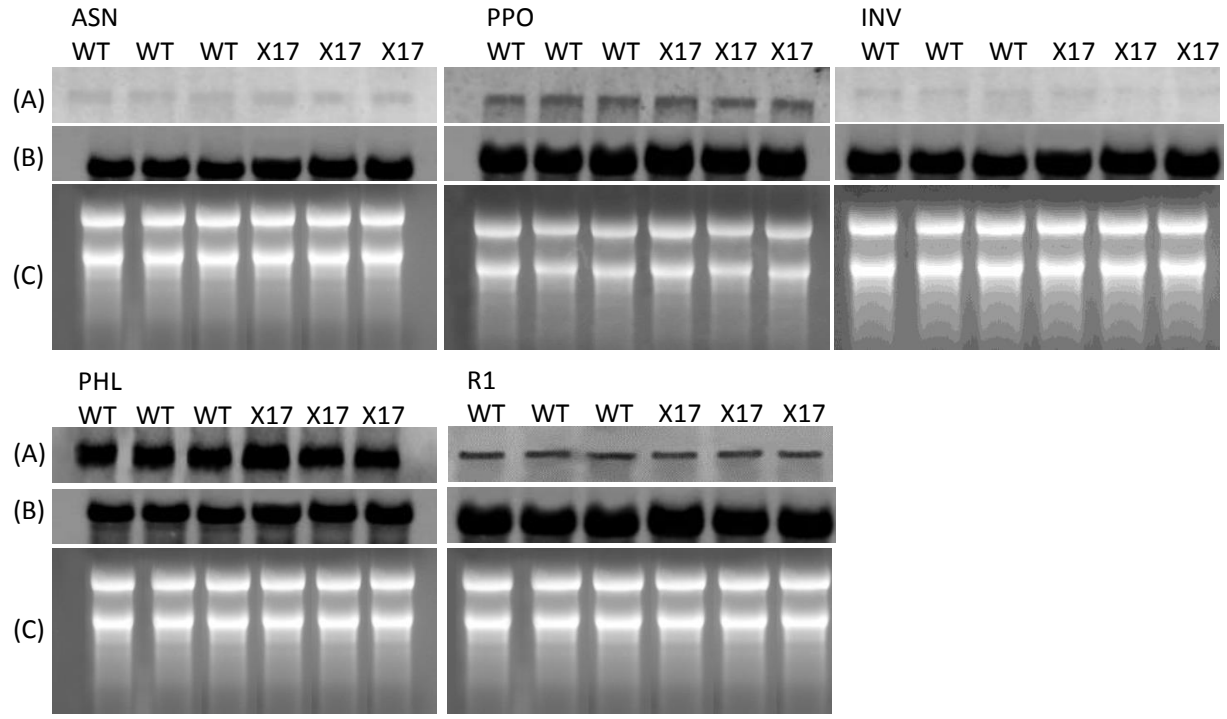


Figure 28. No Effect on Target Transcript Expression in X17 Roots

(A) Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

X17 Flower

Minor decreases in asparagine synthetase and vacuolar invertase transcripts were observed in X17 flower samples (Figure 29). There was no observable down regulation of other target transcripts in X17 flower samples, consistent with down regulation specific to tubers (Figure 29).

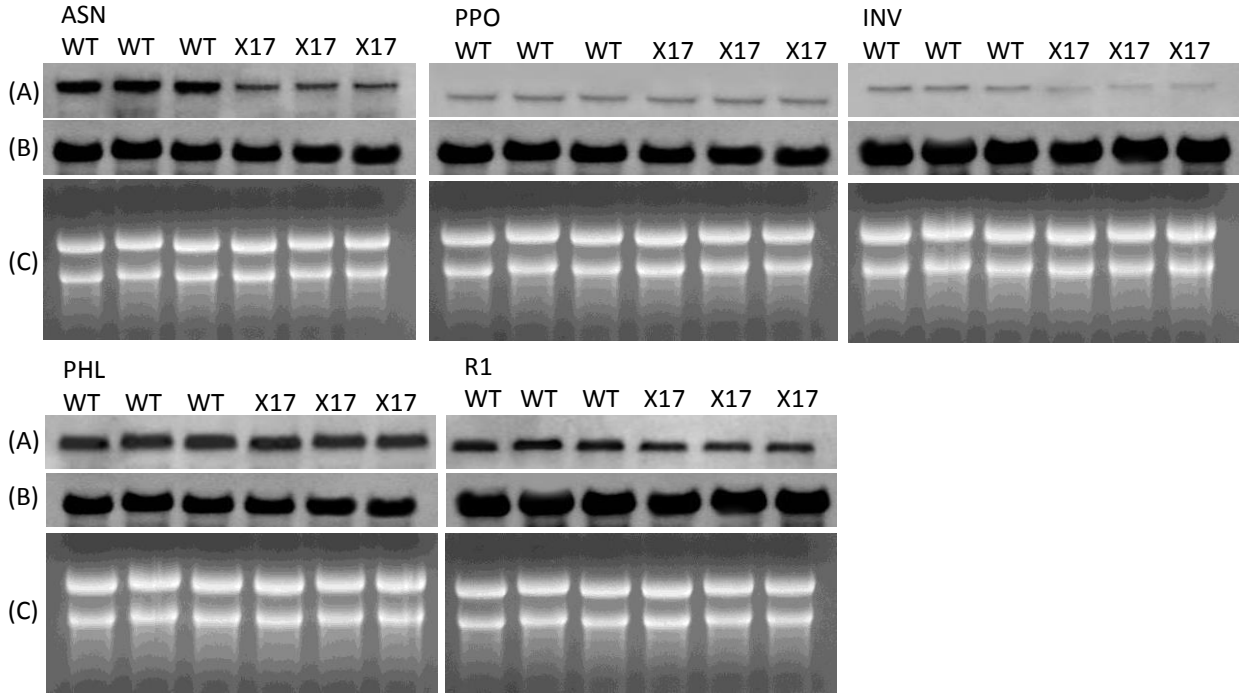


Figure 29. Minor Changes in ASN and VINV Transcript Expression in X17 Flowers

Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (A) Flower samples analysed with the indicated probes. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

Summary of Target Transcript Down Regulation in X17

Northern blots showed down regulation in three of the five transcripts in X17 tubers. Phosphorylase L and water dikinase showed no down regulation. Expression levels in other X17 tissues were unaffected, except for partial down regulation of asparagine synthetase in leaves and asparagine synthetase and vacuolar invertase in flowers.

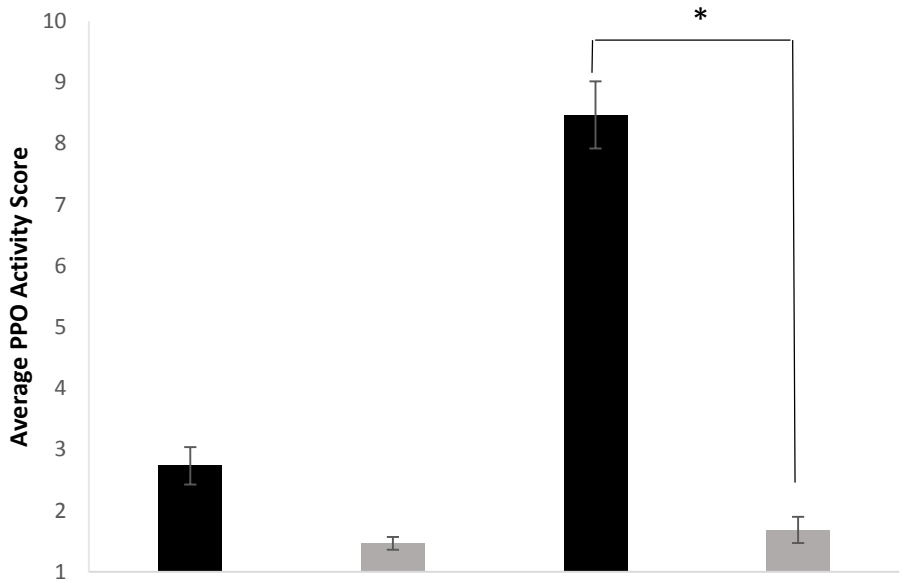
PPO Activity in Event X17

The trait efficacy of polyphenol oxidase (PPO) downregulation in potato event X17 was compared with Ranger Russet (Report15-95-SPS-MOL). A field trial was conducted in Elmore County, Idaho during the 2015 growing season. Plots of X17 and Ranger Russet were harvested, and tubers were assessed for the darkening associated with the PPO enzyme using a colorimetric assay.

PPO activity was measured by monitoring the conversion of an exogenous diphenolic substrate, catechol, into melanin which is associated with a visible colour change. An exogenous substrate was used to increase the rate and extent of colour change in the samples.

Ten tubers from field grown X17 and Ranger Russet were analysed using the PPO activity assay in triplicate.

The colour change associated with each sample was scored using the PPO Activity Colorimetric Scoring Scale with a summary of the results depicted in Figure 30. After 15 minutes, the colour of X17 samples was similar to the T_0 time point, whereas significant colour change occurred in the WT samples after 15 minutes (Figure 30). The difference between the WT and X17 T_{15} samples was statistically significant ($p<0.01$). These results confirm a reduction of PPO activity in X17 tubers.



	WT T_0	X17 T_0	WT T_{15}	X17 T_{15}
Mean	2.733	1.467	8.467	1.683
Std Dev	0.306	0.105	0.549	0.214

Figure 30. Reduced PPO Activity in X17 Compared with Ranger Russet

PPO activity was scored using the PPO Activity Colorimetric Scoring Scale for each of the 10 biological samples. The mean and standard deviation (Std Dev) values are listed above (n=10). The data compares activity scores between X17 and Ranger Russet (WT) at T_0 and T_{15} in the presence of catechol. The average PPO activity score at 15 minutes catechol exposure is significantly lower in X17 when compared with Ranger Russet (* denotes $p<0.01$).

Target Transcript Down Regulation in Y9

Northern blot results showed that asparagine synthetase, polyphenol oxidase, phosphorylase, and vacuolar invertase transcripts were down regulated in tubers while water dikinase transcripts showed no observable down regulation (Figure 31). Partial down regulation was observed for asparagine synthetase in leaves and flowers, and vacuolar invertase in flowers. The 18S rRNA and total RNA levels were consistent across samples allowing direct comparison of asparagine synthetase, polyphenol oxidase, phosphorylase, water dikinase, and vacuolar invertase transcripts between samples in a given tissue. Results are summarised in Table 9. Three biological replicates of Y9 and Atlantic (WT) were evaluated in each gel.

Table 9. Summary of Targeted Down-Regulated Transcripts in Y9 Plants

Transcripts	Tuber	Leaf	Stem	Root	Flower
Asparagine synthetase	✓	✓	-	-	✓
Polyphenol oxidase	✓	-	-	-	-
Phosphorylase L	✓	-	-	-	-
Water dikinase	-	-	-	-	-
Vacuolar invertase	✓	-	-	-	✓

✓ = down regulated, - = not down regulated.

Y9 Tuber

The absence or decreased intensity of bands in the Y9 samples probed for asparagine synthetase, polyphenol oxidase, water dikinase, phosphorylase L, and vacuolar invertase transcripts indicated down regulation in Y9 tubers compared to Atlantic (WT) (Figure 31). The down regulation of asparagine synthetase, polyphenol oxidase, and vacuolar invertase was particularly strong as indicated by the nearly complete absence of the target RNA signal. The down regulation of phosphorylase L was not as strong, but showed decreased expression compared to Atlantic. Water dikinase did not show a decrease in expression compared to Atlantic.

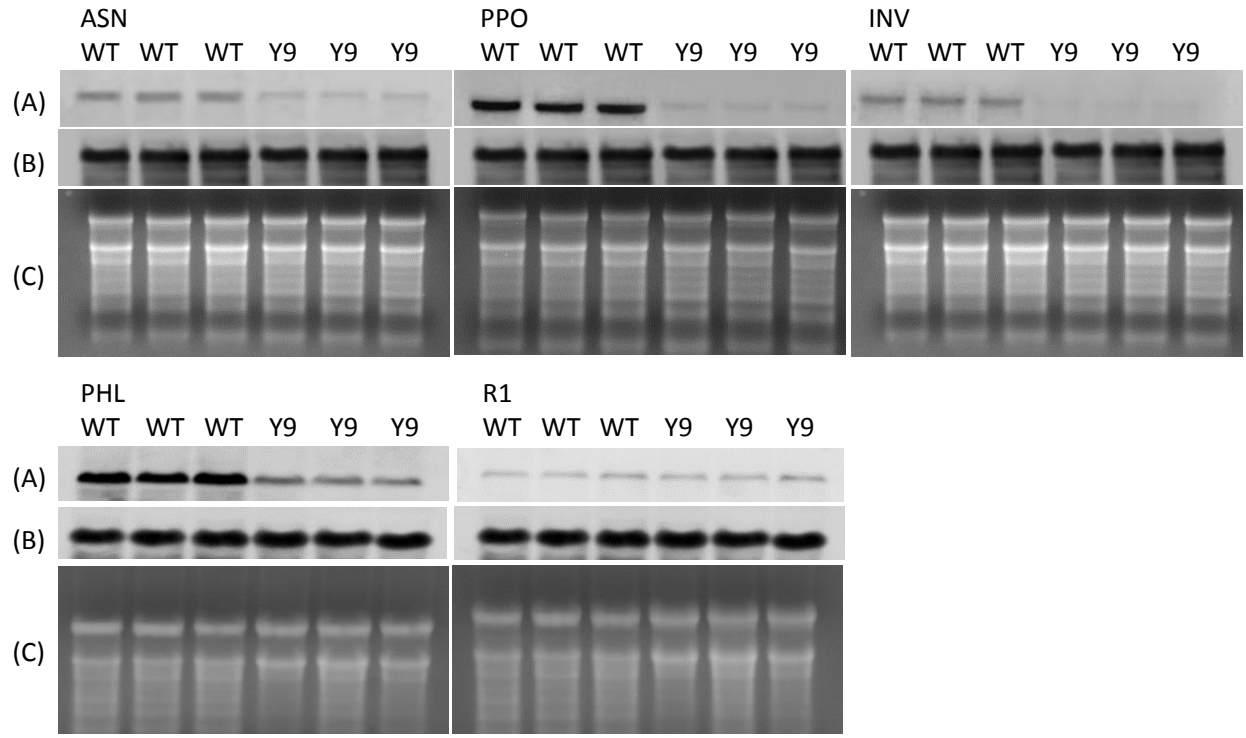


Figure 31. Target Transcript Down Regulation in Y9 Tubers

(A) Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

Y9 Leaf

A decrease in asparagine synthetase transcripts was observed in Y9 leaf samples (Figure 32). There was no observable down regulation of the other transcripts in Y9 leaf samples compared to Atlantic (WT), consistent with tuber-specific down regulation (Figure 32).

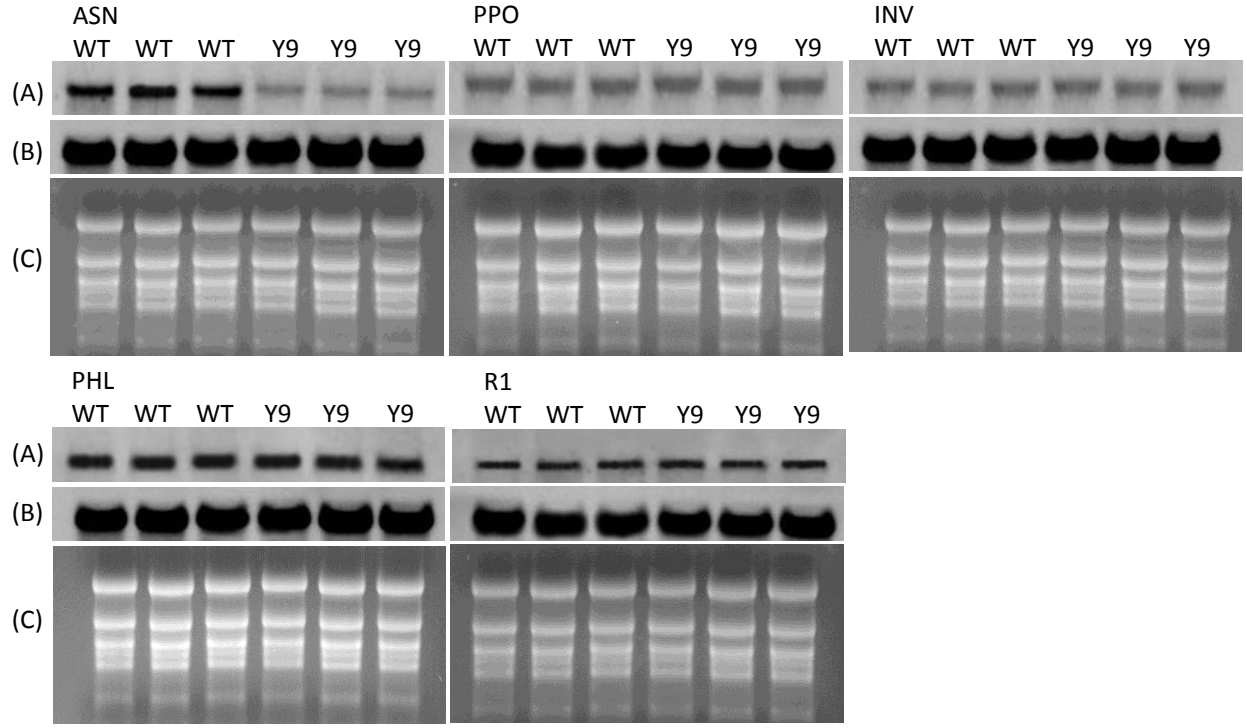


Figure 32. Target Transcript Down Regulation in Y9 Leaves

(A) Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (B) 18S rRNA provide a gel loading control. (C) Total RNA is shown after staining with ethidium bromide. Three biological replicates of Y9 and Atlantic (WT) were evaluated in each gel.

Y9 Stem

Down regulation was not observed for any of the transcripts in Y9 stem samples compared to Atlantic (WT), consistent with down regulation specific to tubers (Figure 33).

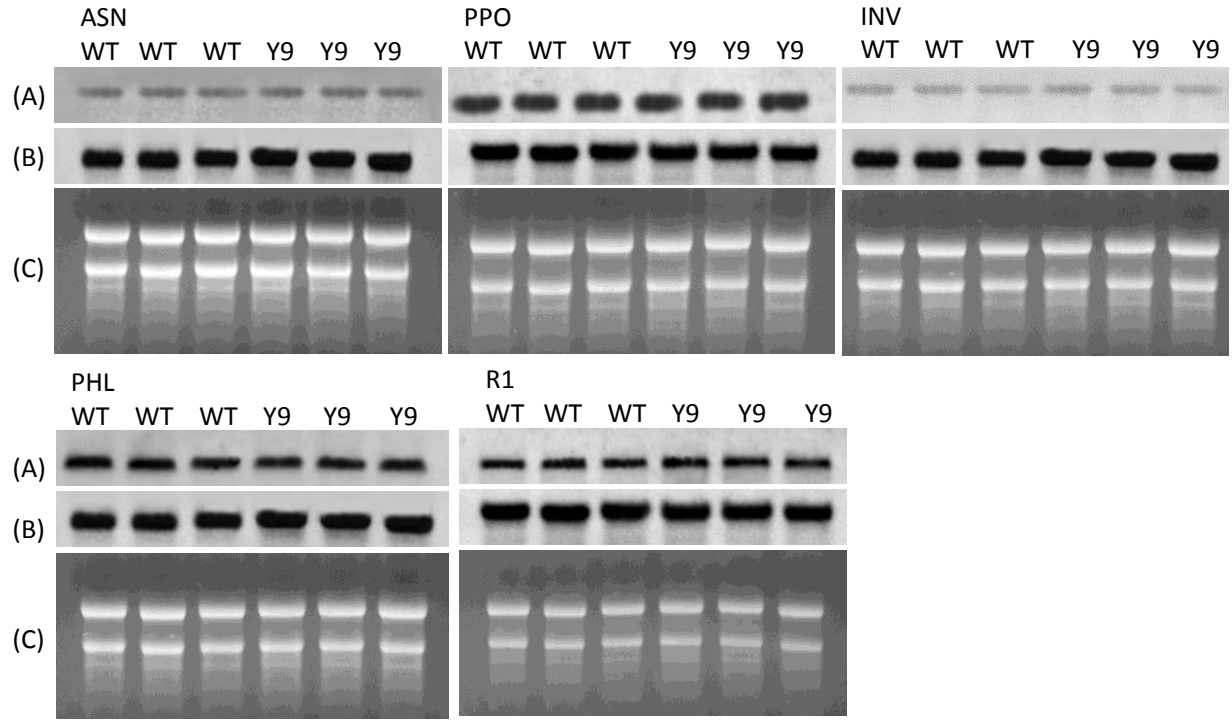


Figure 33. No Effect on Transcript Expression in Y9 Stems

(A) Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide. Three biological replicates of Y9 and Atlantic (WT) were evaluated in each gel.

Y9 Root

Down regulation was not observed for any of the transcripts in Y9 root samples compared to Atlantic (WT), consistent with down regulation specific to tubers (Figure 34).

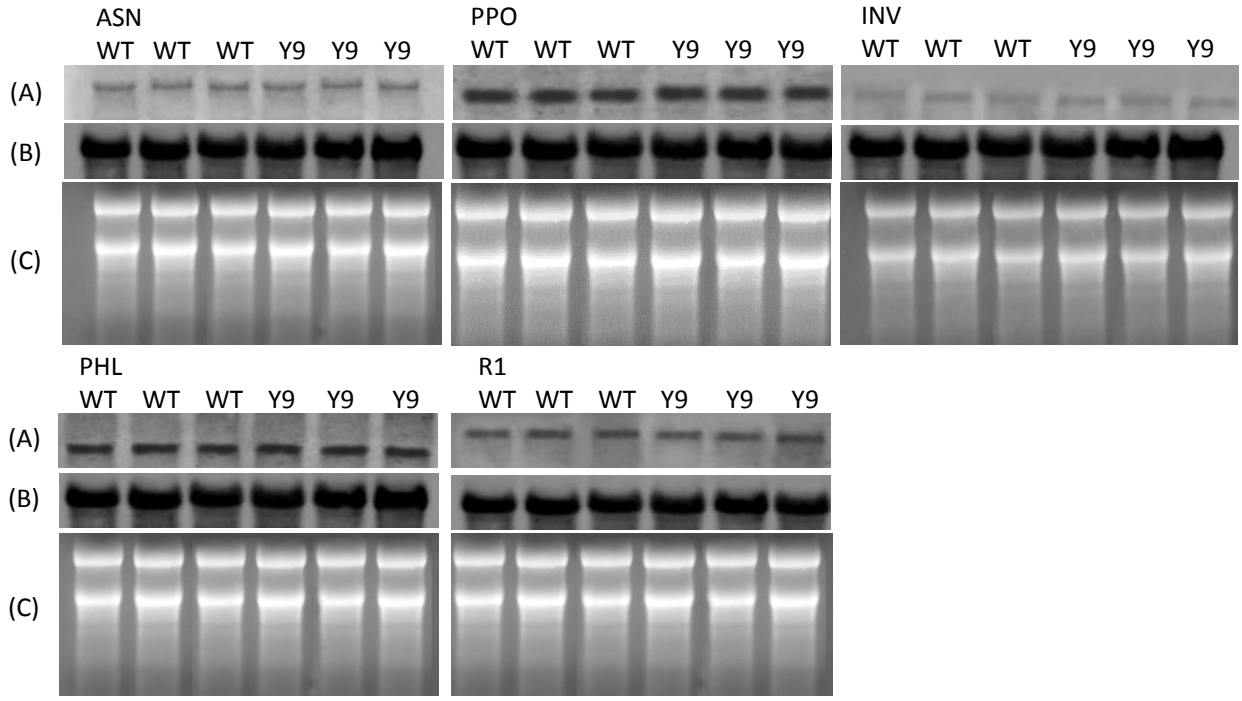


Figure 34. No Observed Transcript Down Regulation in Y9 Roots

(A) Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

Y9 Flower

Minor decreases in asparagine synthetase and vacuolar invertase transcript expression were observed in Y9 flower samples (Figure 35). There was no observable down regulation of other transcripts in Y9 flower samples, consistent with down regulation specific to tubers (Figure 35).

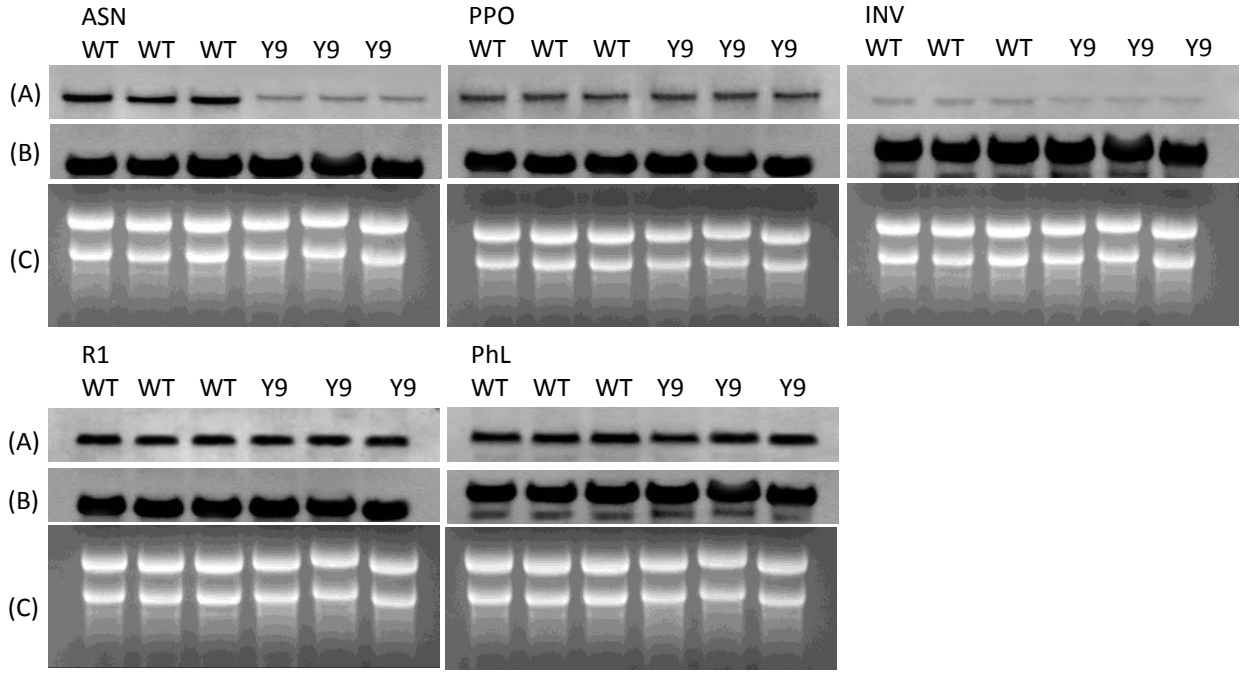


Figure 35. Transcript Down Regulation in Y9 Flowers

(A) Northern blots were hybridised to detect targeted transcripts with the indicated probes: ASN, PPO, PHL, R1, or INV. (B) 18S rRNA provide a gel loading control. (C) Total RNA shown after staining with ethidium bromide.

Summary of Target Transcript Down Regulation in Y9

Northern blot results showed that asparagine synthetase, polyphenol oxidase, phosphorylase L, and vacuolar invertase transcripts were down regulated in tubers while the water dikinase transcripts had no observable down regulation in Y9. Partial down regulation was observed for asparagine synthetase in leaves and flowers, and vacuolar invertase in flowers in Y9.

PPO Activity in Event Y9

The trait efficacy of polyphenol oxidase (PPO) down regulation in potato event Y9 was compared with Atlantic (Report15-96-SPS-MOL). A field trial was conducted in Canyon County, Idaho during the 2015 growing season. Plots of Y9 and Atlantic were harvested, and tubers were assessed for the darkening associated with the PPO enzyme using a colorimetric assay.

PPO activity was measured by monitoring the conversion of an exogenous diphenolic substrate, catechol, into melanin which is associated with a visible colour change. An exogenous substrate was used to increase the rate and extent of colour change in the samples.

Ten tubers from field grown Y9 and Atlantic were analysed using the PPO activity assay in triplicate.

The colour change associated with each sample was scored using the PPO Activity Colorimetric Scoring Scale with a summary of the results depicted in Figure 36. After 15 minutes, the colour of Y9 samples was similar to the T_0 time point, whereas significant colour change occurred in the WT samples after 15 minutes (Figure 36). The difference between the WT and Y9 T_{15} samples was statistically significant ($p<0.01$). These results confirm a reduction of PPO activity in Y9 tubers.

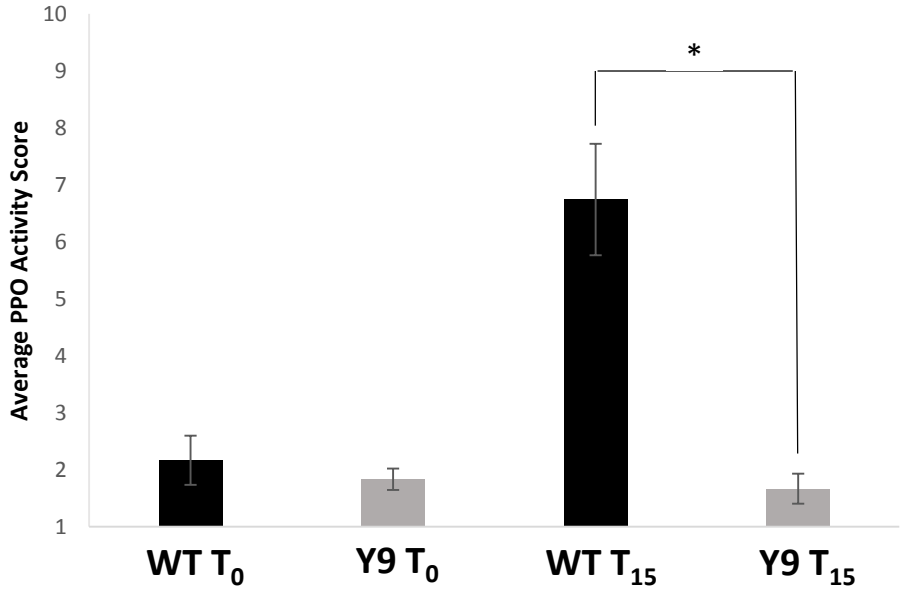


Figure 36. Reduced PPO Activity in Y9 Compared with Atlantic

PPO activity was scored using the PPO Activity Colorimetric Scoring Scale for each of the 10 biological samples. The mean and standard deviation (Std Dev) values are listed above (n=10). The data compares activity scores between Y9 and Atlantic (WT) at T_0 and T_{15} in the presence of catechol. The average PPO activity score at 15 minutes catechol exposure is significantly lower in Y9 when compared with Atlantic (* denotes $p<0.01$).

Conclusion of the Target Transcript Down Regulation of Events W8, X17, and Y9

The *Agp* and *Gbss* promoters are well characterised and are known to be active in tubers and stolons while inducing some transcription in photosynthetically active tissues and roots (Nakata et al., 1994; Visser et al., 1991). Therefore, reduced target mRNA levels mediated by transcription of the inverted repeats were predicted to be strong in tubers, and less pronounced in leaves, stems, and roots. As expected, down regulation of the targeted transcripts was more effective in tubers than in the flowers, roots, stems, or leaves.

The reduced expression of asparagine synthetase, polyphenol oxidase, and vacuolar invertase were consistent with the compositional and trait efficacy data in Sections B.1 and B.5. Although the down regulation of phosphorylase L and water dikinase transcripts was less effective, the intended trait of lower reducing sugars is still prevalent in these events from the down regulation of vacuolar invertase, as shown in the trait efficacy assessment (Section B.5).

Conclusion of the Genetic Characterisation of W8, X17, and Y9

Molecular analyses demonstrated that W8, X17, and Y9 each contained two inserts—one from pSIM1278 and one from pSIM1678. Each insert from pSIM1278 integrated at a single locus in W8, X17, and Y9. Also, each insert from pSIM1678 integrated at a single locus in W8, X17, and Y9, different from the pSIM1278 insert locus.

A combination of Southern blot and DNA sequence analysis was used to determine the structure of each insert in the events. In W8, the pSIM1278 insert contained a nearly full-length T-DNA flanked by a tandem duplication of the Asn1/Ppo5 cassette on the left side and an additional PhL/R1 cassette on the right side. The right side also included a duplication of the *Gbss* promoter with some intervening *PhL* sequence. The pSIM1678 insert contained a structure similar to the pSIM1678 T-DNA, except for a small deletion that includes the LB and part of the *Rpi-vnt1* promoter.

The pSIM1278 insert in X17 consisted of a nearly full-length T-DNA, the same as observed in the parent, F10. Similarly, the pSIM1678 insert in X17 consisted of a nearly full-length T-DNA.

In Y9, the pSIM1278 insert consisted of a nearly full-length pSIM1278 T-DNA flanked by an additional Asn1/Ppo5 cassette on the left side and a partial PhL/R1 cassette on the right side. No differences were observed between the structure of the pSIM1278 insert in Y9 and its J3 parent (Table 5). The pSIM1678 insert consisted of a nearly full-length T-DNA with an additional partial VInv cassette on the right side.

The inserts in W8, X17, and Y9 consisted solely of the sequences targeted for insertion and did not contain any detectable backbone sequence. The studies confirmed the stability of the DNA inserts in W8, X17, and Y9 across multiple cycles of vegetative propagation, which demonstrated that inserts likely will be maintained through vegetative propagation.

B. Characterisation and Safety Assessment of New Substances

B.1. Characterisation and Safety Assessment of New Substances

B.1(a) a full description of the biochemical function and phenotypic effects of all new substances (e.g. a protein or an untranslated RNA) that are expressed in the new GM organism, including their levels and site of accumulation, particularly in edible portions

Late blight protection was achieved by expression of the VNT1 protein in W8, X17, and Y9. VNT1 specifically recognises the *P. infestans* secreted Avr-vnt1 effector, enabling the plant to initiate its immune response (Pel, 2010). The immune response acts through a conserved network of signaling pathways and induces a hypersensitive response (Coll et al., 2011; Panstruga et al., 2009). The hypersensitive response destroys infected plant tissue through programmed cell death, restricting growth and spread of the pathogen.

The pSIM1678 vector includes an expression cassette for the *Rpi-vnt1* gene originating from *Solanum venturii*. The gene product, VNT1, is an R-protein involved in the plant hypersensitive response that protects potato against late blight infection from *P. infestans*.

Expression of the *Rpi-vnt1* mRNA in W8, X17, and Y9 is driven by its native promoter and terminator. Quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) was used to verify gene expression of the *Rpi-vnt1* transcript in W8, X17, and Y9 leaves and tubers. Total RNA was isolated from tissues of W8, X17, Y9, and their respective control varieties and subjected to RT-qPCR using *Rpi-vnt1* specific primers. Gene expression of *Rpi-vnt1* was normalised to a set of endogenous housekeeping genes, α-tubulin, and elongation factor 1α, within each sample.

The expression of VNT1 in events W8, X17, and Y9 is summarised with full details presented in the following studies:

- Report15-70-SPS-MOL, W8;
- Report15-09-SPS-MOL, X17; and
- Report15-17-SPS-MOL, Y9.

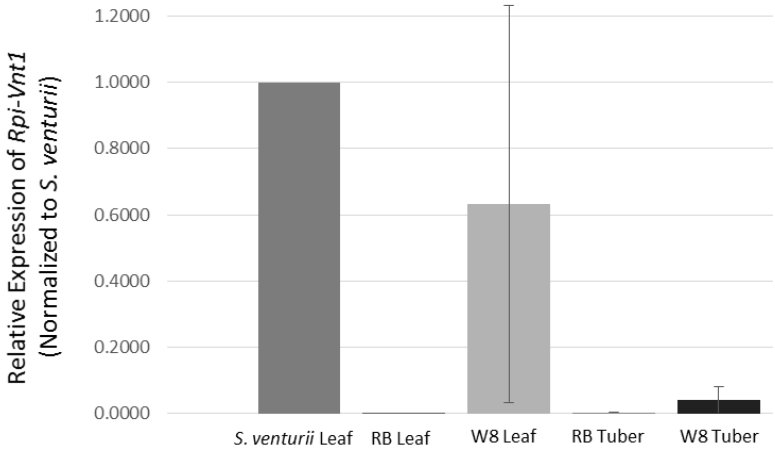
Further, the efficacy of the *Rpi-vnt1* gene in late blight protection is demonstrated.

VNT1 Expression in Events W8, X17, and Y9

Gene expression studies showed *Rpi-vnt1* was expressed similarly in the foliar tissue of W8, X17, Y9, and the native *S. venturii*, but found much lower expression in W8, X17, and Y9 tubers than in leaves (Report15-70-SPS-MOL; 15-09-SPS-MOL; 15-17-SPS-MOL). A sensitive, immunoblot assay was unable to detect VNT1 protein in W8, X17, or Y9 tissues with a sensitivity of 30 and 60 ppb in tubers and leaves, respectively. A conservative estimate for the levels of VNT1 in W8, X17, and Y9 tissues was established to be less than 100 ppb.

***Rpi-vnt1* Transcript Expression**

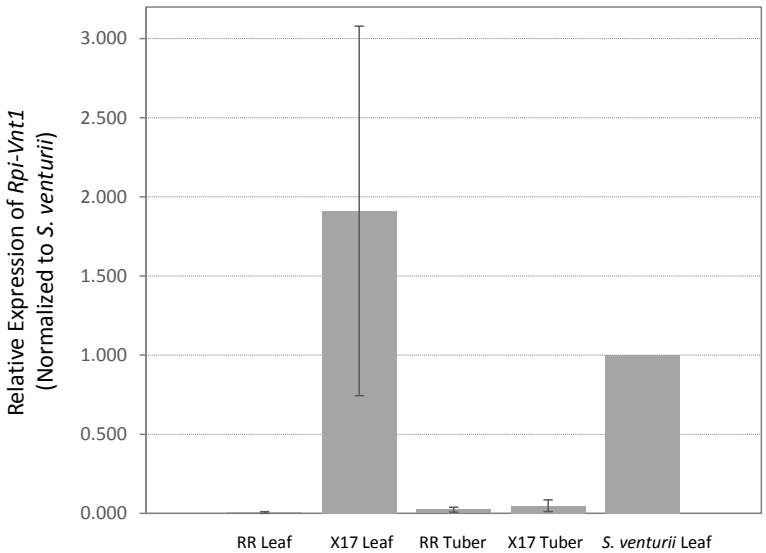
Rpi-vnt1 transcript levels in Russet Burbank and W8 leaves were compared to levels in the native *S. venturii* leaves. Expression levels of the *Rpi-vnt1* gene were similar between W8 and *S. venturii* leaves (Figure 37). Expression of the *Rpi-vnt1* gene in Russet Burbank leaves was not detected. Significantly lower levels of *Rpi-vnt1* expression were measured in W8 tubers compared to W8 leaves. This pattern of expression, higher in leaves and lower in tubers, is consistent with other work on R-gene expression (Pel, 2010).



Mean		0.0002	0.6333	0.0026	0.0390
S.D.		6×10^{-5}	0.0862	0.0023	0.0215

Figure 37. *Rpi-vnt1* Transcript Levels in W8 Measured by RT-qPCR

W8 and Russet Burbank (RB) tuber and leaf assays were performed on three biological replicates analysed in triplicate. Data were normalised to endogenous reference genes, *Elongation Factor 1α* and *α-Tubulin*, and then all normalised expression was set relative to *S. venturii*.



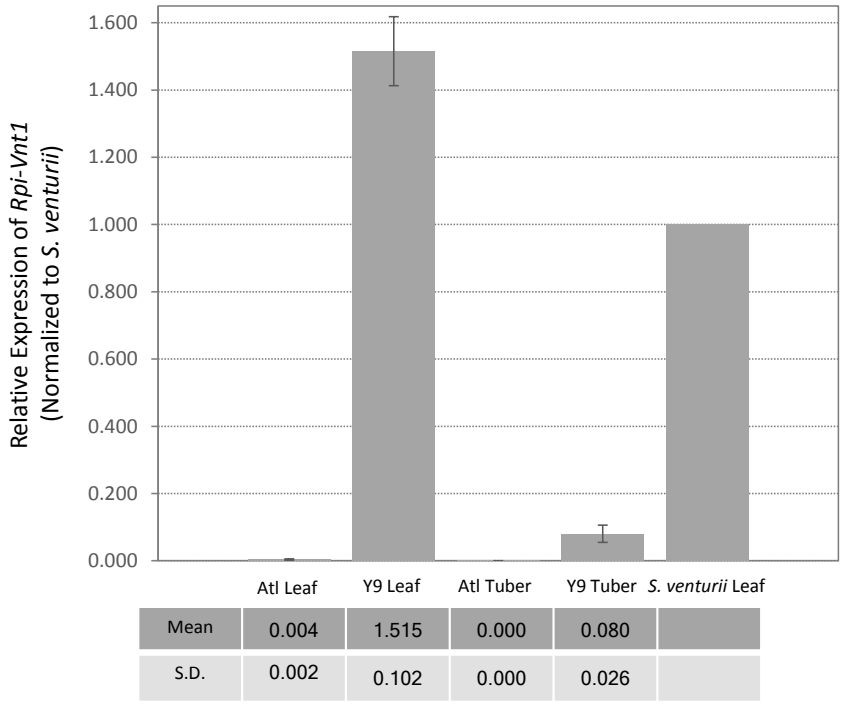
Mean	0.006	1.912	0.024	0.049	
S.D.	0.006	1.168	0.016	0.037	

Figure 38. *Rpi-vnt1* Transcript Levels in X17 Measured by RT-qPCR

X17 and Ranger Russet (RB) tuber and leaf assays were performed on three biological replicates analysed in triplicate. Data were normalised to endogenous reference genes, *Elongation Factor 1α* and *α-Tubulin,* and then all normalised expression was set relative to *S. venturii*.

Rpi-vnt1 transcript levels in Ranger Russet and X17 leaves were compared to levels in the native *S. venturii* leaves. Expression levels of the *Rpi-vnt1* gene were similar between X17 and *S. venturii* leaves (Figure 38). Expression of the *Rpi-vnt1* gene in Ranger Russet leaves was not detected. Significantly lower levels of *Rpi-vnt1* expression were measured in X17 tubers compared to X17 leaves. The pattern of expression, higher in leaves and lower in tubers, is consistent with other work on R-gene expression (Pel, 2010).

Rpi-vnt1 transcript levels in Atlantic and Y9 leaves were compared to levels in the native *S. venturii* leaves. Expression levels of the *Rpi-vnt1* gene were similar between Y9 and *S. venturii* leaves (Figure 39). Expression of the *Rpi-vnt1* gene in Atlantic leaves was not detected. Significantly lower levels of *Rpi-vnt1* expression were measured in Y9 tubers compared to Y9 leaves. The pattern of expression, higher in leaves and lower in tubers, is consistent with other work on R-gene expression (Pel, 2010).



	Atl Leaf	Y9 Leaf	Atl Tuber	Y9 Tuber	*S. venturii* Leaf
Mean	0.004	1.515	0.000	0.080	
S.D.	0.002	0.102	0.000	0.026	

Figure 39. *Rpi-vnt1* Transcript Levels in Y9 Measured by RT-qPCR

Y9 and Atlantic (Atl) tuber and leaf assays were performed on three biological replicates analysed in triplicate. Data were normalised to endogenous reference genes, *Elongation Factor 1α* and *α-Tubulin,* and then all normalised expression was set relative to *S. venturii*.

VNT1 Protein Expression

The gene product, VNT1, is an 891 amino acid R-protein of the coiled-coil (CC), nucleotide-binding site (NBS), and leucine-rich repeat (LRR) class involved in the plant hypersensitive response, and protects potato against late blight infection from *Phytophthora infestans* (Foster et al., 2009). Numerous R-protein homologs are present in potato and tomato varieties and other wild *Solanum* species (Jupe et al., 2012). R-proteins are expressed at low levels in plants, and in some cases are estimated to be as low as 18 parts per trillion, leading to negligible human exposure (3.6 ng protein/year) (Bushey et al., 2014).

An antibody-based method was developed for the detection and quantification of VNT1 (Report15-70-SPS-MOL). Multiple approaches were used to produce antibodies against VNT1. The most sensitive polyclonal

antibody was produced against the FHSSSKLPFGVWESKIL peptide from the LRR domain. This antibody was used for western blot studies attempting to detect and quantitate the VNT1 protein in W8, X17, and Y9 tissues. The limit of detection (LOD) for the antibody was established using the *E. coli* expressed VNT1-LRR protein (i.e. MPB-LRR fusion), as it could be purified, while attempts to purify *N. benthamiana* expressed VNT1 were unsuccessful. VNT1-LRR co-purified with the *E. coli* GroEL chaperone protein (Figure 40A) and represented about 33% of the total protein concentration based upon densitometry. A serial dilution of VNT1-LRR was used to establish an LOD of about 9 pg (Figure 40B).

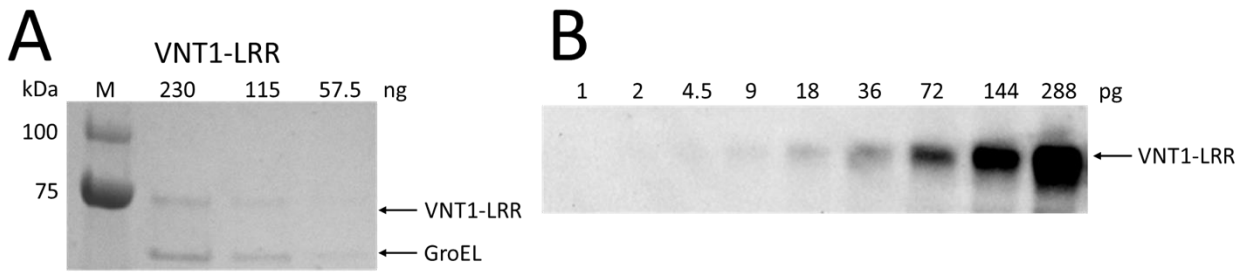


Figure 40. Limit of Detection of Anti-VNT1 Antibody

(A) Densitometric analysis of Coomassie-stained SDS-PAGE gel was used to determine the concentration of the purified VNT1-LRR from *E. coli* as about 33% of the total protein. (B) Western blot analysis was used to determine an LOD for the western blot assay as 9 pg of purified VNT1-LRR.

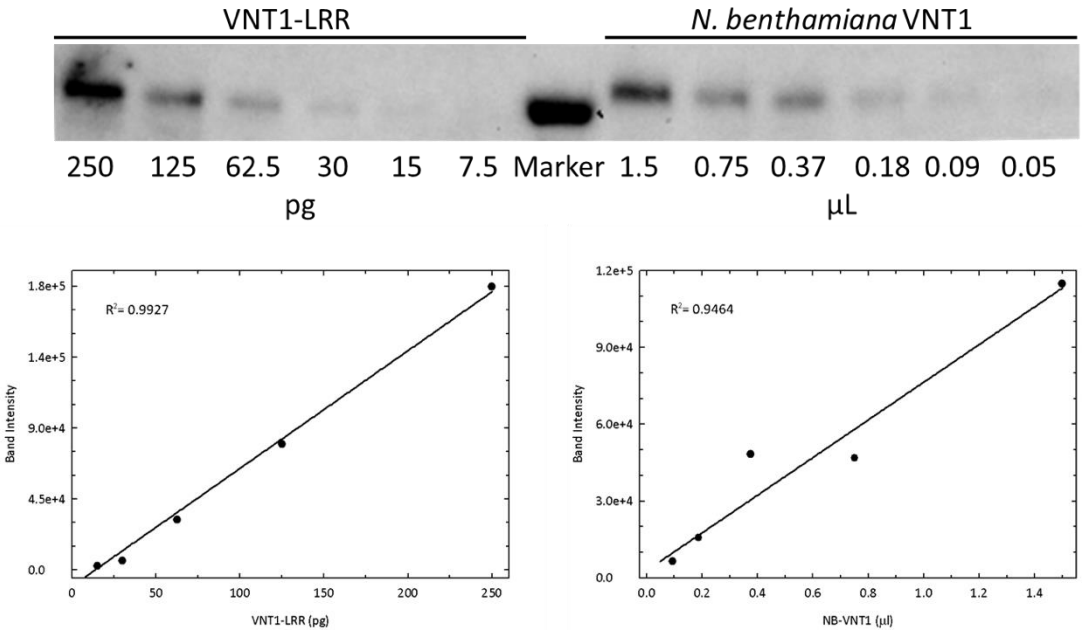


Figure 41. Estimation of NB VNT1 Concentration

Linear regression of the western blot band intensities was used to determine the concentration of VNT1 within the *N. benthamiana* sample (NB VNT1). The resulting equation for VNT1-LRR was y = 746.97x - 10,210 and for NB VNT1 was y = 73,528x + 2,886. The total amount of NB VNT1 in 1 µl was calculated to be 116 ± 10 pg.

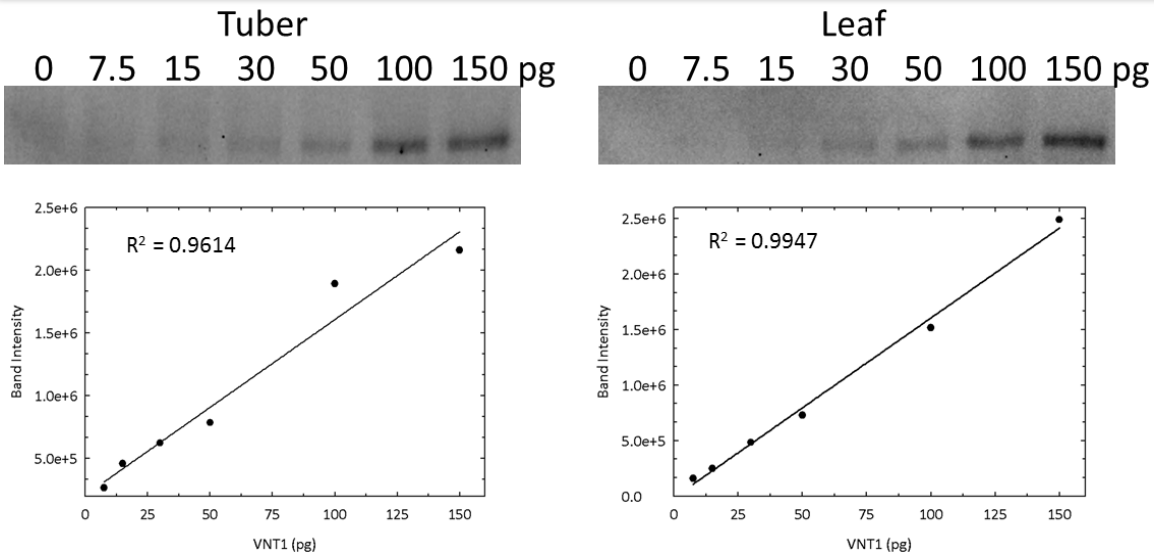


Figure 42. Limit of Quantitation of VNT1 Protein in Russet Burbank Tissues

Protein extracts from 1 mg tuber or 0.5 mg leaf were spiked with 0, 7.5, 15, 30, 50, 100, and 150 pg full-length *N. benthamiana* expressed VNT1 (NB VNT1). Standard curves show the assay is within the linear dynamic range. LOQ values of 30 and 60 ppb were determined for tubers and leaves, respectively (e.g. 30 pg/0.5 mg = 60 ppb).

The concentration of VNT1 protein in *N. benthamiana* (NB VNT1) samples was determined using the standard curve of VNT1-LRR from *E. coli* (Figure 41). The limit of quantification (LOQ) was determined using a western blot of serially diluted NB VNT1 protein spiked into Russet Burbank protein extracts (Figure 42). An LOQ of 30 and 60 ppb was established in tuber and leaf samples, respectively.

Detection of VNT1 Protein in Events W8, X17, Y9

Protein extracts of W8, X17, Y9, Russet Burbank, Ranger Russet, and Atlantic tuber and leaf samples were analysed using western blot assays (Figure 43; Figure 44; Figure 45). Although the antibody could detect VNT1 at low ppb concentrations, cross-reactivity was high in tuber and leaf samples, probably due to the many VNT1 homologs in Russet Burbank, Ranger Russet and Atlantic. This was more pronounced in samples containing high concentrations of total protein (i.e. 20 µg) required for measurements near the LOQ. High protein concentrations were loaded into the wells in order to detect the very low concentrations of VNT1.

VNT1 was not detectable in W8, X17, or Y9 tuber or leaf samples, so it was concluded that the concentration of VNT1 in these samples was below the LOQ. A conservative estimate for the levels of VNT1 in W8, X17, and Y9 tissues was established to be less than 100 ppb.

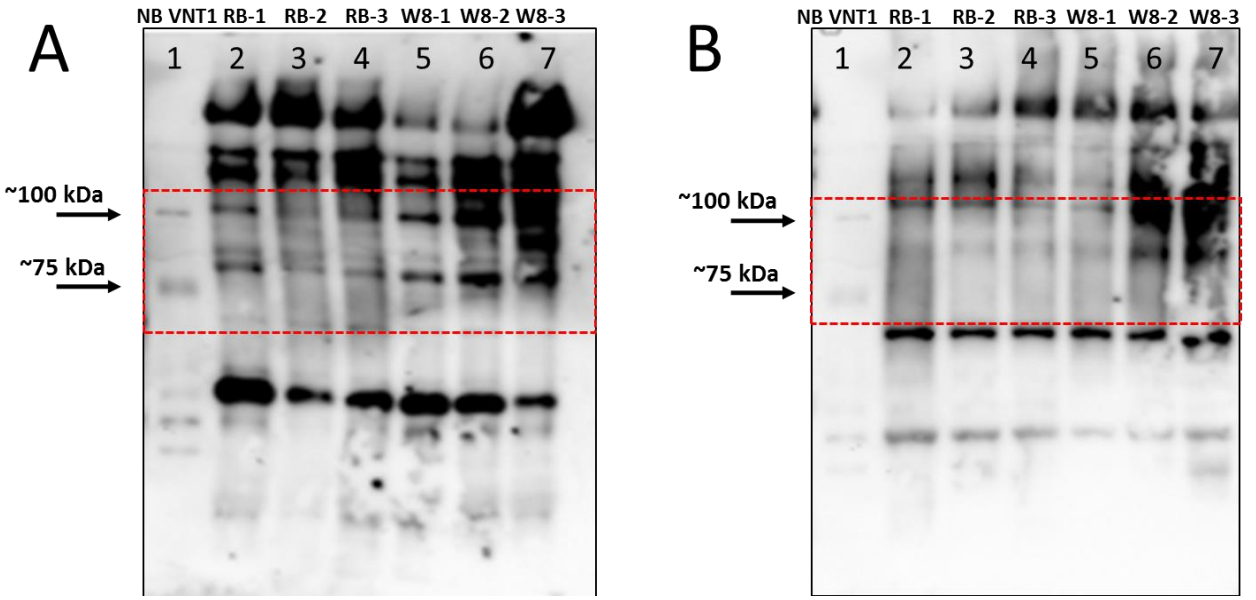


Figure 43. VNT1 Limit of Quantitation in W8

Total protein extracts from Russet Burbank and W8 tubers (A) or leaves (B) were separated by SDS-PAGE and transferred to PVDF membranes for immunodetection. Lane 1, NB VNT1 (97 pg) appeared as approximately 100 kDa full-length and about 75 kDa processed form (arrows); Lanes 2-4, Russet Burbank (RB); Lanes 5-7, W8. Dashed red boxes indicate regions of expected VNT1 migration based upon NB VNT1 samples.

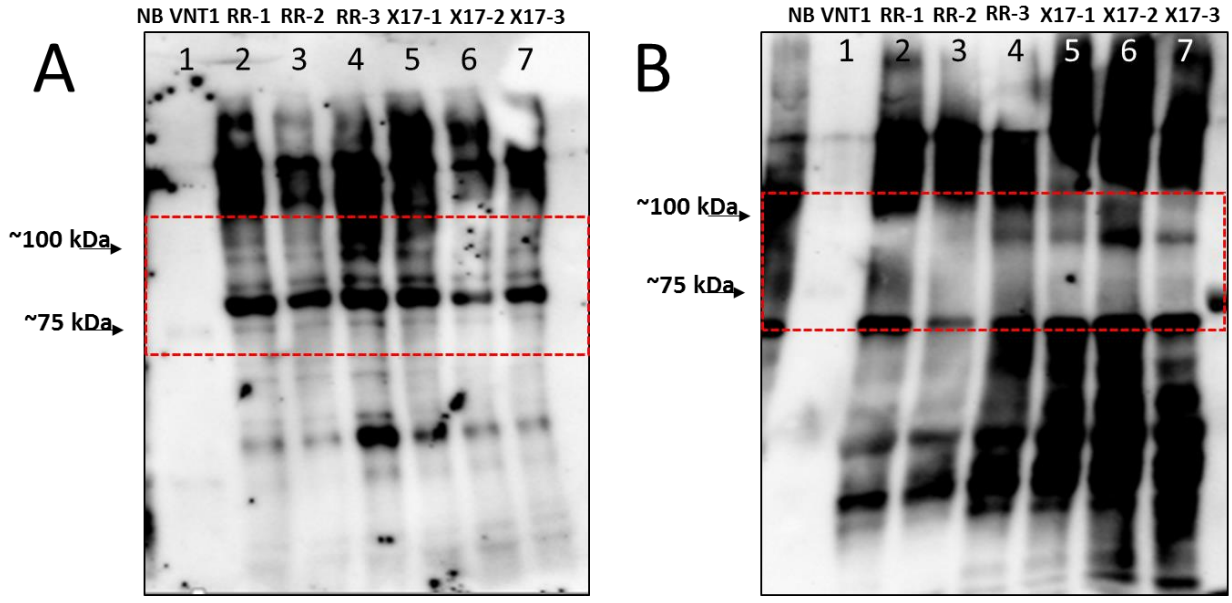


Figure 44. VNT1 Limit of Quantitation in X17

A. Total protein extracts from Ranger Russet and X17 tubers were separated by SDS-PAGE and transferred to PVDF membranes for immunodetection. **B.** Total protein extraction from Ranger Russet and X17 leaves. Lane 1, NB VNT1 (97 pg) appears as about 100 kDa full-length and approximately 75 kDa processed form (arrows); Lanes 2-4, Ranger Russet (RR); Lanes 5-7, X17. Dashed red boxes indicate regions of expected VNT1 migration based upon NB VNT1 samples.

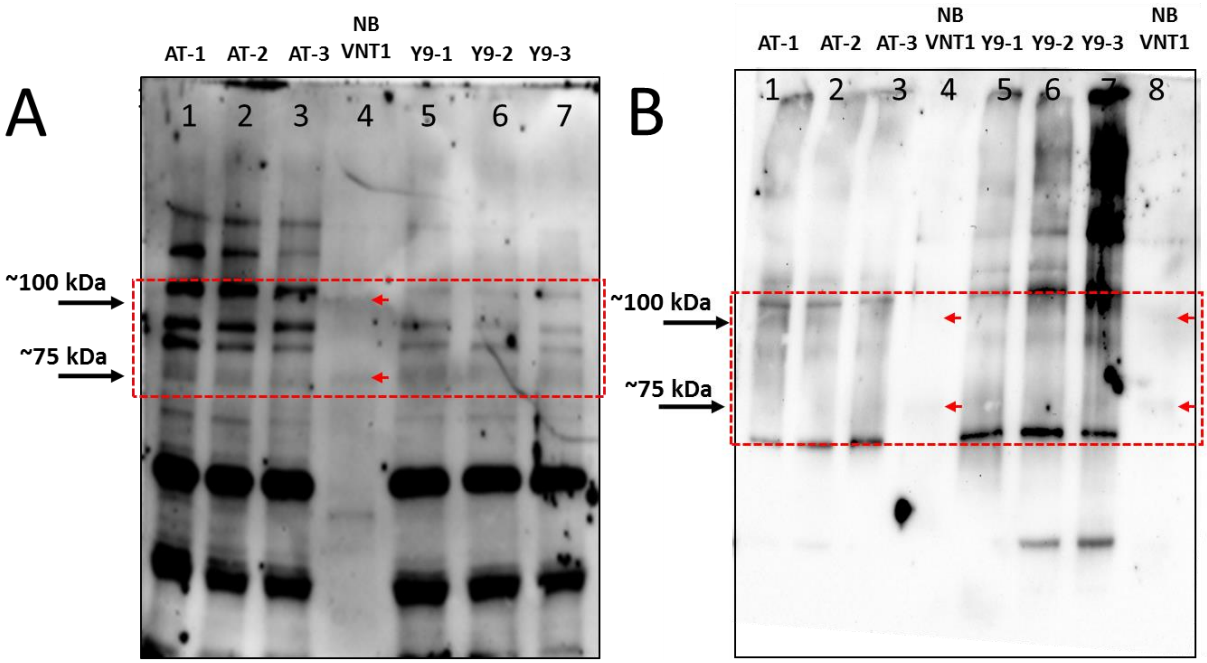


Figure 45. VNT1 Limit of Quantitation in Y9

Total protein extracts from Atlantic and Y9 tubers (**A**) or leaves (**B**) were separated by SDS-PAGE and transferred to PVDF membranes for immunodetection. Lane 4, 8, NB VNT1 (97 pg) appears as approximately 100 kDa full-length and about 75 kDa processed form (arrows); Lanes 1-3, Atlantic (AT); Lanes 5-7, Y9. Dashed red boxes indicate regions of expected VNT1 migration based upon NB VNT1 samples.

Late Blight Efficacy in Events W8, X17, and Y9

The *Rpi-vnt1* gene in W8, X17, and Y9 confers foliar protection against certain strains of late blight. Efficacy was evaluated against four late blight strains: US-8, US-22, US-23, and US-24. All four strains are important in North America (Hwang et al., 2014; Wijekoon et al., 2014). The methods for these studies are provided in Report 13-04-SPS-ENV, Report 14-04-SPS-ENV, and Report 16-72-SPS-ENV.

Two years of field trials were conducted to evaluate the field efficacy of W8, X17, and Y9 against late blight (*P. infestans*) compared to the non-transformed control varieties. In 2013 and 2014, test and control plants were grown at three locations Table 10 and Table 11).

Table 10. Late Blight Efficacy Field Study Sites in 2013

Site State	Site County	Trial Design[1]	Planting Date	Inoculation Date	Inoculum Strain
Idaho	Boundary	RCB, 4 reps	10 May 2013	1 Aug 2013	US-8
Michigan	Ingham	RCB, 4 reps	1 May 2013	26 Jul 2013	US-22 and US-23
Pennsylvania	Centre	RCB, 4 reps	12 Jun 2013	8 Aug 2013	US-23

[1]RCB=Randomised Complete Block design (number of blocks was equal to the number of reps)

Table 11. Late Blight Efficacy Field Study Sites in 2014

Site State	Site County	Trial Design[1]	Planting Date	Inoculation Date	Inoculum Strain
Michigan	Ingham	RCB, 4 reps	13 Jun 2014	25 Jul 2014	US-23
Pennsylvania	Centre	RCB, 4 reps	7 Jul 2014	Not applicable	US-23[2]
North Dakota	Cass	RCB, 3 reps	4 Jun 2014	15, 18, 22 Aug 2014[3]	US-24

[1]RCB=Randomised Complete Block design (number of blocks was equal to the number of reps)
[2]At the Pennsylvania site, natural infection of *P. infestans* was observed, and the disease was evenly distributed across the research farm. To avoid introducing different mating types of pathogens into the field, no inoculation was done. Isolates of the pathogen from the trial were sent to Cornell University on August 18, 2014 for genotyping. The genotype of the pathogen at our farm was confirmed to be US 23.

Foliage affected over time was recorded (Figure 46 to Figure 50) and the area under the disease progress curve (AUDPC) was calculated. AUDPCs are relative measurements of late blight infection over the course of the growing season. The greater the area, the greater the infection. AUDPC was analysed statistically to determine the effect of the potato late blight resistance gene in W8, X17, and Y9. The measured AUDPC for each event and the control variety are summarised in Table 12 through Table 19.

A significant reduction in late blight foliar infection was observed in W8, X17, and Y9 compared to their control varieties, demonstrating field efficacy of the late blight protection trait.

Field Efficacy of Late Blight Protection in W8, X17, and Y9 in 2013

A significant reduction in foliar late blight infection was observed in each event when compared to the control varieties (Table 12, Table 13, and Table 14). These findings demonstrated the field efficacy of the *Rpi-vnt1* gene in W8, X17, and Y9. The percent foliage affected by late blight over time after inoculation at each site is shown in Figure 46, Figure 47, and Figure 48. Greater variability was observed at the Michigan site, but efficacy was still demonstrated.

Table 12. AUDPC for W8 across 2013 Sites

Variety	N	Mean AUDPC	Standard Deviation	P-value[1]
W8	12	5.83	12.3	**<0.0001**
Russet Burbank		1502	855	

[1]P-values indicating significant differences between test and control are bold and underlined.

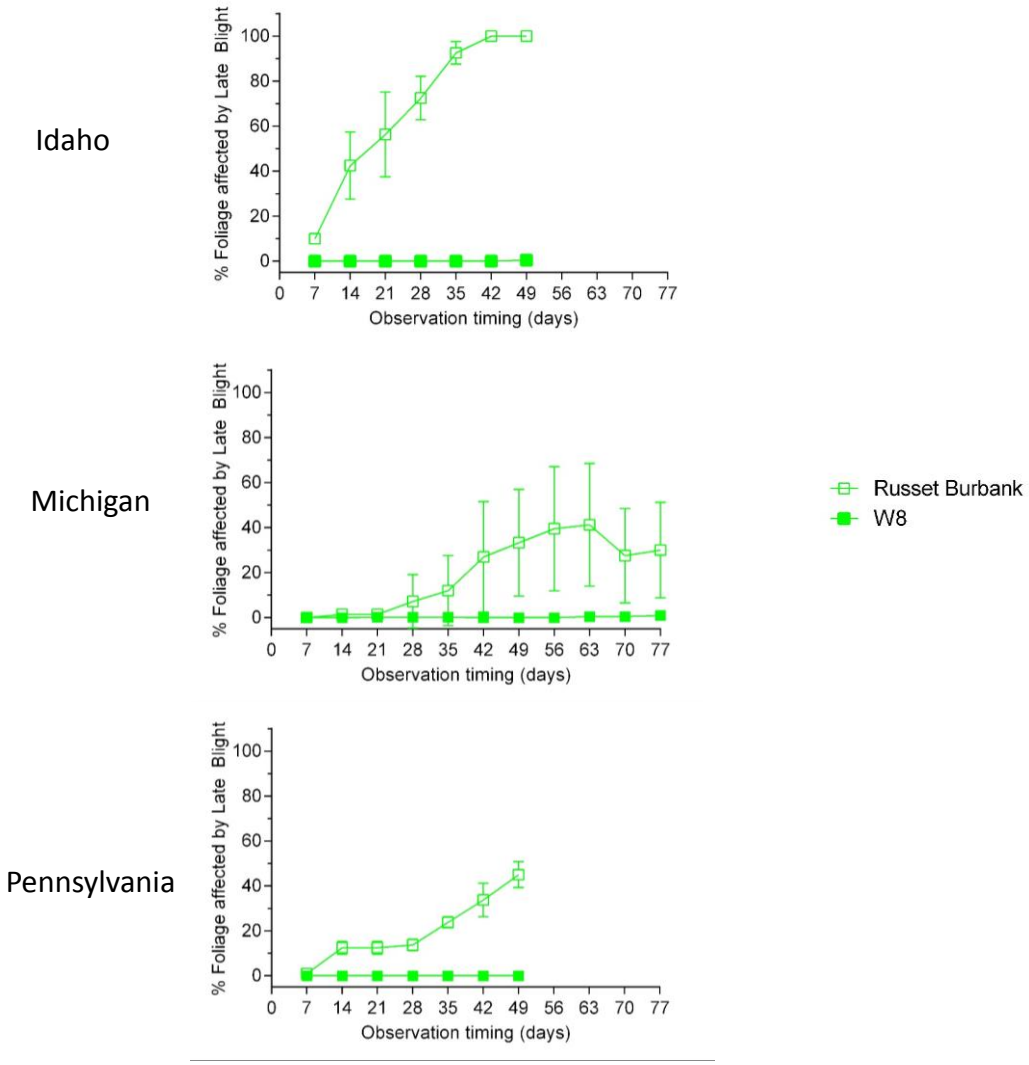


Figure 46. Percent W8 and Russet Burbank Foliage Affected by Late Blight at 2013 Field Sites

Table 13. AUDPC for X17 across 2013 Sites

Variety	N	Mean AUDPC	Standard Deviation	P-value[1]
X17	12	26.0	60.9	**<0.0001**
Ranger Russet		2207	733	

[1]P-values indicating significant differences between test and control are bold and underlined.

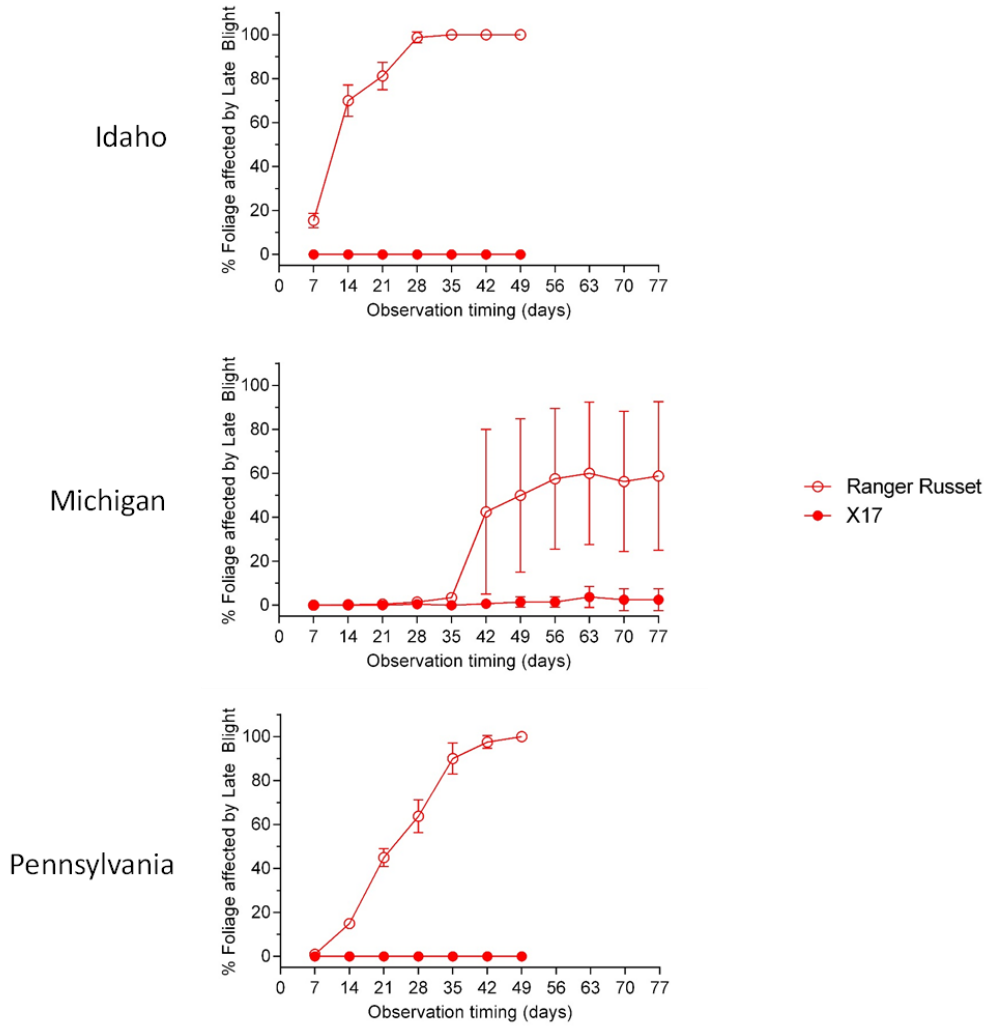


Figure 47. Percent Ranger Russet and X17 Foliage Affected by Late Blight at 2013 Field Sites

Table 14. AUDPC for Y9 across 2013 Sites

Variety	N	Mean AUDPC	Standard Deviation	P-value[1]
Y9	12	0.583	2.02	**<0.0001**
Atlantic		2453	563	

[1]P-values indicating significant differences between test and control are bold and underlined.

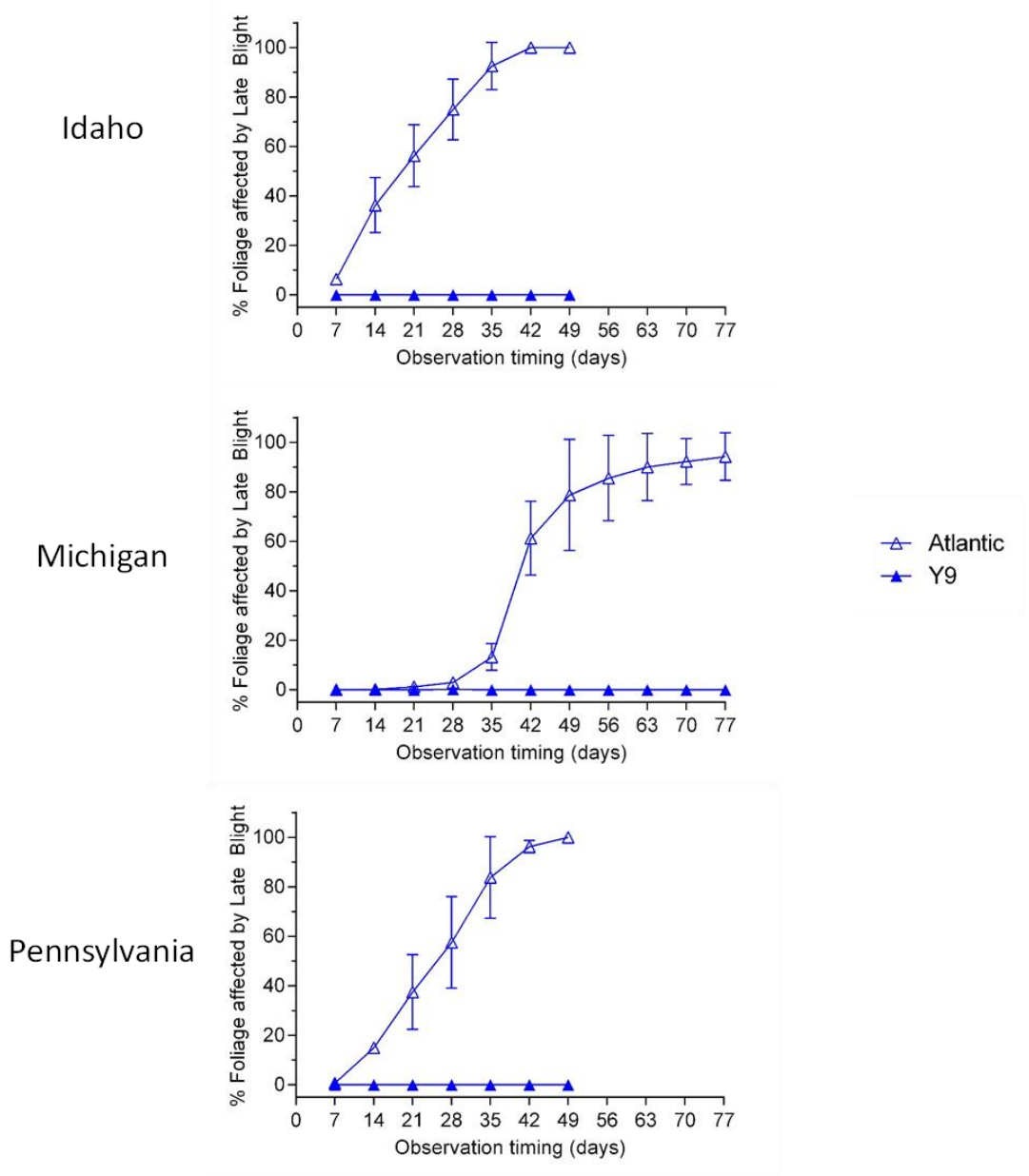


Figure 48. Percent Atlantic and Y9 Foliage Affected by Late Blight at 2013 Field Sites

Field Efficacy of Late Blight Protection in W8, X17, and Y9 in 2014

A significant reduction in foliar late blight infection was observed in each event when compared to the control varieties (Table 15, Table 16, Table 17, Table 18 and Table 19). These findings demonstrated the field efficacy of the *Rpi-vnt1* gene in W8, X17, and Y9. The percent foliage affected by late blight over time at each site is shown in Figure 49, Figure 50, and Figure 51.

Table 15. AUDPC for W8 across 2014 Michigan and Pennsylvania Sites

Variety	Mean AUDPC	Standard Deviation	P-value[1]
W8	1.75	1.87	**<u><0.0001</u>**
Russet Burbank	1666	316	

N=8

[1]P-values indicating significant differences between test and control are bold and underlined.

Table 16. AUDPC for W8 at 2014 North Dakota Site

Variety	Mean AUDPC	Standard Deviation	P-value[1]
W8	338	56	**<u><0.0001</u>**
Russet Burbank	1924	93	

[1]P-values indicating significant differences between test and control are in bold and underlined

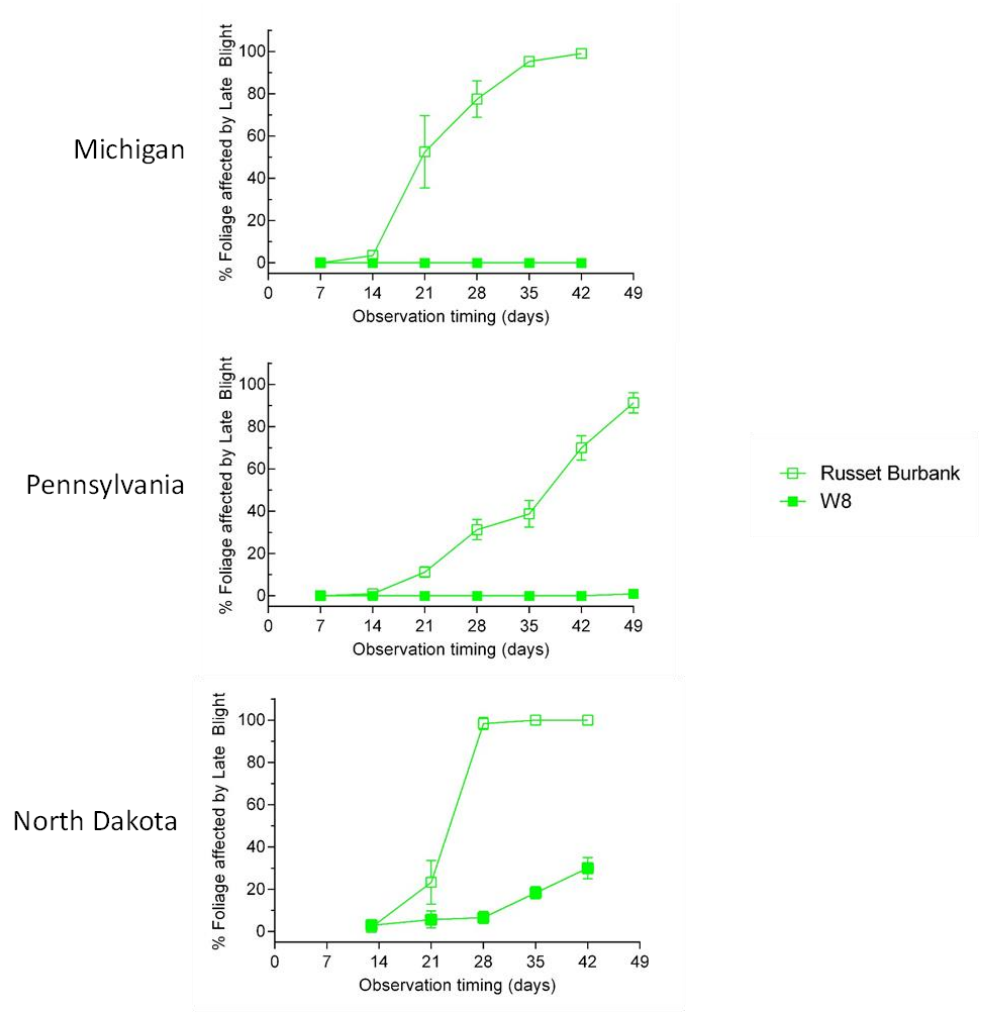


Figure 49. Percent Russet Burbank and W8 Foliage Affected by Late Blight at 2014 Field Sites

Table 17. AUDPC for X17 across 2014 Michigan and Pennsylvania Sites

Variety	Mean AUDPC	Standard Deviation	P-value[1]
X17	0.438	1.24	**<u><0.0001</u>**
Ranger Russet	1968	286	

N=8

[1]P-values indicating significant differences between test and control are bold and underlined.

Table 18. AUDPC for X17 in 2014 North Dakota Site

Variety	Mean AUDPC	Standard Deviation	N	P-value[1]
X17	357	90	3	**<u><0.0001</u>**
Ranger Russet	1964	192	3	

[1]P-values indicating significant differences between test and control are in bold and underlined.

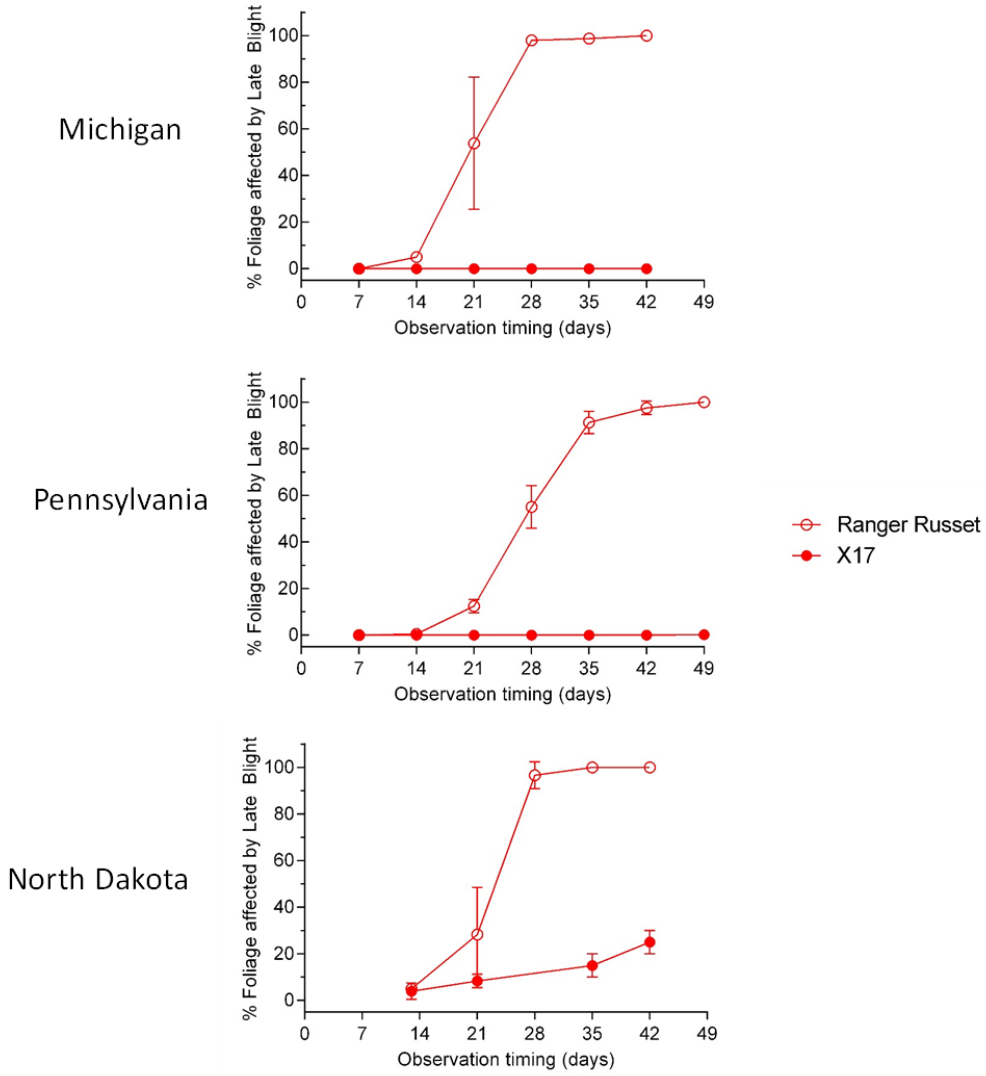


Figure 50. Percent Ranger Russet and X17 Foliage Affected by Late Blight at 2014 Field Sites

Table 19. AUDPC for Y9 across 2014 Michigan and Pennsylvania Sites

Variety	Mean AUDPC	Standard Deviation	P-value[1]
Y9	3.06	4.75	**<0.0001**
Atlantic	1838	354	

N=8

[1]P-values indicating significant differences between test and control are bold and underlined.

Table 20. AUDPC for Y9 at 2014 North Dakota Site

Variety	Mean AUDPC	Standard Deviation	P-value[1]
Y9	602	87	**<0.0001**
Atlantic	2187	208	

[1]P-values indicating significant differences between test and control are in bold and underlined

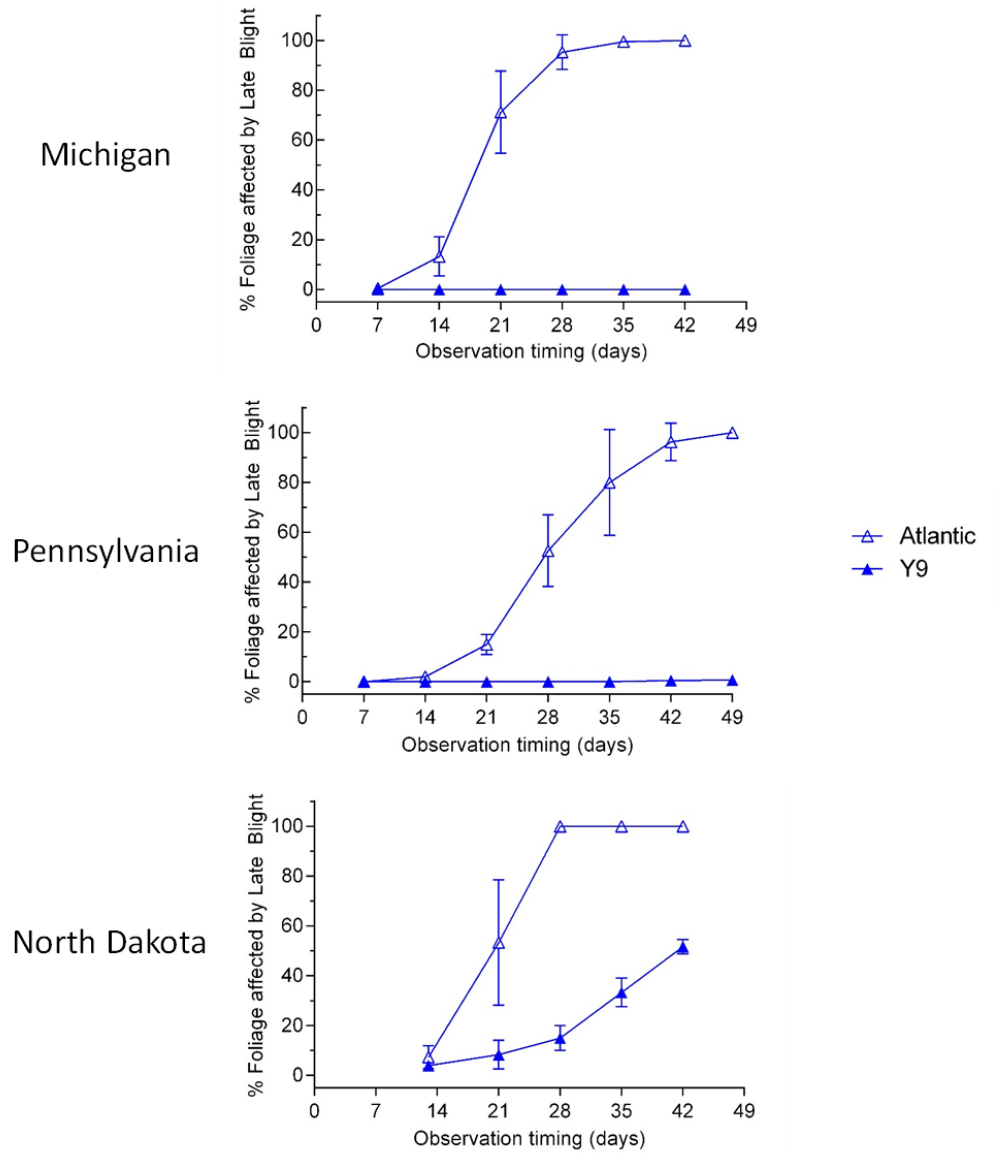


Figure 51. Percent Atlantic and Y9 Foliage Affected by Late Blight at 2014 Field Sites

Summary of Late Blight Efficacy

The field efficacy of the late blight protection trait in W8, X17, and Y9 against US-8, US-22, US-23, and US-24 strains of *P. infestans* was evaluated in field trials. A significant reduction in late blight foliar infection was observed in W8, X17, and Y9 compared to their control varieties, demonstrating field efficacy of the late blight protection trait.

B.1(b) Information about prior history of human consumption of the new substances, if any, or their similarity to substances previously consumed in food.

Safety Assessment of the VNT1 Protein

A weight-of-evidence approach using risk assessment principles was used to evaluate the safety of the VNT1 protein. This approach considers all data in a comprehensive manner to evaluate the safety of VNT1, including risk assessment results (potential hazard X potential exposure = potential risk). The safety assessment of the VNT1 protein was based on the following considerations:

- The *Rpi-vnt1* gene source has a history of safe use;
- VNT1 belongs to a class of proteins ubiquitous throughout plants;
- VNT1 activates the plant's native immune response pathway;
- VNT1 lacks significant homology to known allergens and toxins;
- VNT1 is homologous to proteins with a history of safe use; and
- The potential exposure of humans and livestock to VNT1 is negligible.

Based on the weight-of-evidence and taking into account the close-to-zero risk, the VNT1 protein in events W8, X17, and Y9 is a safe as conventional varieties for humans, livestock, and the environment.

The Source of VNT1 has a History of Safe Use

The events W8, X17, and Y9 contain the *Rpi-vnt1* gene from *S. venturii.* The coding sequence of this gene is 100% identical to the *Rpi-phu1* gene from *S. phureja* (Foster et al., 2009). *S. phureja* is used as a source of genetic material in traditional breeding to improve potato varieties and has an established history of safe use (OECD, 1997). *S. phureja* is a diploid potato originating from the Andean valleys of South America and is grown throughout the central region of Bolivia (Gabriel et al., 2013).

Breeding programs are underway in Europe and South America to introgress *Rpi-vnt1* into commercial potatoes (Coca-morante and Tolín-tordoya, 2013; Gabriel et al., 2013). For example, the *Rpi-vnt1* gene has been introgressed into *S. tuberosum* from an interspecific cross between a *S. phureja* derived clone and the potato cultivar Sárpo Mira (Tomczyńska et al., 2014). In addition, the *Rpi-vnt1* gene has been introgressed into *S. tuberosum* by crossing with a hybrid between *S. phureja* and *S. stenotomum*, known as pinta boca, in Peru and Bolivia (Śliwka et al., 2010). The *Rpi-vnt1* gene is found in both *S. venturii* and *S. phureja*, so the introduction of *Rpi-vnt1* into W8, X17, and Y9 is expected to have similar safe use.

VNT1 Belongs to a Class of Proteins Ubiquitous Throughout Plants

To infect a host plant, *P. infestans* penetrates the plant and translocates protein effectors into the cells. These effectors can interfere with defense processes directly or can disrupt host-signaling mechanisms. To combat pathogens, plants have evolved the capacity to recognise specific pathogen effectors by intracellular protein receptors encoded by R-genes.

The protein products of R-genes (R-proteins) have a conserved tripartite domain organization consisting of a central nucleotide-binding site (NBS) flanked by either an N-terminal toll-like/interleukin-1 (TIR) or CC domain, and a C-terminal LRR domain. The LRR domain is the least conserved of the three domains and is involved in protein-protein interactions and ligand binding of pathogen effectors (Mchale et al., 2006).

Numerous and ancient in origin, plant NBS-LRR proteins are encoded by one of the largest gene families known in plants (Mchale et al., 2006). These proteins are present in early plant lineages (Chiang and Coaker, 2015). Moreover, there are many NBS loci in plant genomes. In potato, approximately 435 NBS-LRR encoding genes were annotated when the genome sequence of *S. tuberosum* group *phureja* was analysed for the presence of R-genes (Lozano et al., 2012). However, due to the potato's highly heterozygous nature and tetraploid genome, it has been estimated that cultivated potato could contain thousands of R-genes (Lozano et al., 2012).

Other widely consumed plant species that contain R-genes encoding proteins with the conserved tripartite domain organisation are listed in Table 21. The prevalence of plant NBS-LRR R-protein coding genes demonstrates that the VNT1 protein is similar to proteins commonly present in the food supply.

Table 21. NBS-LRR Genes are Ubiquitous in Crops

Crop	Approximate Number of NBS-LRR Genes	Reference
Potato (*S. tuberosum* group *phureja*)	435	Lozano et al., 2012
Maize (*Zea mays*)	150	Song et al., 2015
Soybean (*Glycine max*)	319	Kang et al., 2012
Rice (*Oryza sativa* L.)	400	Monosi et al., 2004
Apple (*Malus x domestica Borkh*)	900	Velasco et al., 2010
Grape (*Vitis vinifera*)	459	Marone et al., 2013

Decades of consumption of commercial potatoes with R-proteins demonstrates a history of safe use of R-proteins. In addition to widespread occurrence of R-proteins in potatoes and other commonly consumed foods, some commercial potato varieties possess R-genes effective against *P. infestans* from wild solanum species. For example, the late blight resistance genes *Rpi-blb2* from *S. bulbocastanum, R1-R5, R8, and R10* from *S. demissum, Rpi-Mcd1* from *S. microdontum,* and *Rpi-ber* from *S. berthaultii* have been incorporated into commonly consumed commercial potatoes (Tan et al., 2008; Vleeshouwers et al., 2011; Vossen et al., 2016). The presence of R-genes in commonly consumed foods (Table 21) and in commercial potato varieties (Lozano et al., 2012) demonstrates a history of safe use.

VNT1 Activates the Plant's Native Immune Response Pathway

Characterising the mode of action for VNT1 in potato and the systems in which it interacts informs the evaluation of hazard potential for risk assessment. R-proteins are maintained in an inactive state at low concentrations in the cell, and are activated by pathogen-secreted effector proteins (Avr proteins) (Spoel and Dong, 2012).

VNT1 specifically recognises the *P. infestans* secreted Avr-vnt1 effector, enabling the plant to activate its immune response (Pel, 2010). The immune response acts through a conserved network of signalling pathways that induce a hypersensitive response (Coll et al., 2011; Panstruga et al., 2009). The hypersensitive response destroys infected plant tissue through programmed cell death, restricting growth and spread of the pathogen.

Although recognition of Avr-vnt1 is specific to VNT1, the activated immune response pathway triggering the hypersensitive response exists commonly in plants (Coll et al., 2011; Marone et al., 2013). Sequence and structural similarities between R-gene receptors from different plant species, and similarities in observed immune responses, demonstrate that R-proteins generally activate conserved defence pathways in plants (Baker et al., 1997; Feys and Parker, 2000).

The VNT1 protein shares highly similar structural and functional properties with other R-proteins that are widely distributed in nature. VNT1 activates the plant's own immune response pathway, already present in the cell. Protection against disease is the only function so far demonstrated for R- proteins (Mchale et al., 2006). The conserved structure and function of R-proteins and their wide distribution in nature (Leipe et al., 2004) demonstrate that VNT1 triggers existing cellular pathways and is safe to introduce into food crops.

VNT1 Lacks Significant Homology to Known Allergens and Toxins

Using established bioinformatic tools, VNT1 was assessed for similarity to known allergens and toxins according to guidelines from Codex (2003 and FAO/WHO (2001). The analytical methods are described in Report16-47-SPS-MOL. An allergen search was conducted to identify matches between the VNT1 protein sequence and known allergens compiled in the 2016 AllergenOnline.org database, available through the Food Allergy Resource Research Program (FARRP) via the University of Nebraska. For toxin homology searches, the NCBI database was queried using all protein sequences annotated with the keyword “toxin” (Entrez query: “toxin”; E-value < 10^{-2}).

Analysis of the full-length VNT1 protein did not identify any allergen matches that satisfied the search parameter threshold (E-value < 10^{-4}). The full-length VNT1 protein has no significant sequence identity with known or suspected allergens. Furthermore, the 8-mer exact match search did not identify any matches with a potential allergen.

The toxin search did not identify any protein toxins with significant homology to VNT1. The search identified a number of protein records containing the keyword “toxin”. Each protein match was examined further and is described in Report 16-47-SPS-MOL. None of the matches were known toxins, proteins with toxic properties, or proteins with likely toxic potential.

In summary, based on a review of established bioinformatic sources, no significant sequence homology exists between the VNT1 protein and known allergens or toxins. The VNT1 protein does not share sequence or structural similarity with proteins known to pose toxicological and allergenic concerns.

VNT1 is Homologous to Proteins with a History of Safe Use

Proteins with a history of safe use, or that are structurally and functionally related to proteins with a history of safe use, generally are considered safe to consume (Hammond and Cockburn, 2008). As a component of the safety assessment of VNT1, bioinformatic analyses were conducted to identify sequence homology between the VNT1 protein and proteins with a history of safe use.

R-proteins are expressed at very low levels in plants and in one case were estimated to be as low as 18 parts per trillion (ppt) (Bushey et al., 2014). In addition, homologous R-proteins are found in potato with high sequence similarity (Table 22), making it challenging to detect or quantify the VNT1 protein in potato tissues.

Table 22. Similarity of VNT1 to Other R-Proteins from *S. tuberosum* (Potato)

R-protein	Source	Accession No.	% identical amino acids
VNT1	*S. venturii* (wild solanum species)	ACJ66594.1	100
St-Tm-2 ToMV	*S. tuberosum* (potato)	ABM05492.1	77
RPP13-like protein 3	*S. tuberosum* (potato)	XP_015170548.1	79
RPP13-like protein 3	*S. tuberosum* (potato)	XP_006360919.1	80

RPP13-like protein	*S. tuberosum* (potato)	XP_006360932.1	87

The similarity of the S. venturii VNT1 amino acid sequence to potato R-proteins is shown for four cultivated varieties with the highest sequence homology (Table 22). The potato St-Tm-2 ToMV R- protein shares about 77% sequence identity with VNT1. The three potato RPP13-like proteins share from 79 to 87% sequence identity with the VNT1 protein.

Six R-proteins from cultivated tomato and pepper varieties that share the highest amino acid sequence homology to the S. venturii VNT1 are shown in Table 23. These five tomato R-proteins and one chili pepper R-protein (Lanfermeijer et al., 2003; Mueller et al., 2005) share 74 to 77% sequence identity with the VNT1 protein expressed in W8, X17, and Y9.

Table 23. High Sequence Similarity between VNT1 and Other R-proteins

Protein	Accession No.	% Identical amino acids to VNT1	Binomial	Common name
VNT1	ACJ66594.1	100	*Solanum venturii*	Wild solanum species
Tm-2 ToMV	AAQ10735.1	76	*Solanum lycopersicum*	Tomato
Tm-2 ToMV	AAV87531.1	75	*Solanum lycopersicum*	Tomato
Tm-2^2 ToMV	AAQ10736.1	76	*Solanum lycopersicum*	Tomato
tm-2	AAQ10734.1	76	*Solanum lycopersicum*	Tomato
RPP-13 like protein	XP_004247881.1	77	*Solanum lycopersicum*	Tomato (Heinz 1706)
RPP13-like protein 3	XP_016566165.1	74	*Capsicum annuum*	Pepper

Bioinformatic analysis demonstrated that VNT1 protein has high sequence similarity to ten characterized or putative R-proteins with a long history of safe use. The VNT1 protein shares about 74 to 87% sequence identity with these proteins. All of these genes and proteins are commonly consumed in cultivated potato, tomato, and pepper cultivars, providing additional evidence that VNT1 is as safe for human consumption as R-proteins in other foods.

Low Dietary Exposure of VNT1 to Humans in the USA

An estimate of dietary exposure of humans to VNT1 was calculated assuming tubers contained less than 100 ppb of VNT1, using United States potato consumption data. North American (including United States) potato consumption rates are the second highest compared to other regions of the world (Table 24). Therefore, United States potato consumption data provide conservative (high-end) estimates of dietary exposure to VNT1 in W8, X17, and Y9 potatoes.

Dietary exposure calculations were determined using a conservative estimate of potato consumption in the United States of 35 kg per capita (DEEM-FCID). By comparison, in Australia and New Zealand the estimated potato consumption is 35.53 kg and 22.89 kg per capita, respectively (Fresh Logic, 2014; Potatoes NZ, 2014). Therefore, United States exposure estimates are comparable to potato consumption in Australia and New Zealand.

Table 24. Potato Consumption by Geographic Region

Region	Potato consumption (kg/capita/year)[1]
Africa	14
Asia/Oceania	24
Europe	88
Latin America	21
North America[2]	58

[1]Food and Agriculture Organization of the United Nations (FAO, 2008).
[2]United States average consumption is approximately 35 kg/ capita /year (DEEM-FCID).

Human dietary exposure to VNT1 was calculated using the 95th percentile United States potato consumption from the Dietary Exposure Evaluation Model–Food Commodity Intake Database (DEEM-FCID; EPA, 2014). The calculations used consumption levels of people who eat more potatoes and potato-containing products than 95% of the population (Table 25). In the DEEM model, potato consumption includes a wide variety of potato sources, such as whole tuber (which includes fries, hash browns, etc.), chips, dry flakes, flour, and components of potatoes present in infant food. While the consumption data in the DEEM-FCID model are derived from a United States survey, the data apply to other populations. Consumption of potato in Africa, Asia/Oceania and Latin America is lower than in North America, and Europe has the highest per capita potato consumption (FAO, 2008).

Based on DEEM exposure (95th percentile), the United States total population has a potato consumption rate of 2.85 g/kg body weight/day (kg bw/d) (Table 25). The sub-population with the highest consumption of potatoes is children aged 1-2, with 7.14 g/kg bw/d (Table 25). The potential exposure of humans to VNT1 in W8, X17, and Y9 potatoes was evaluated by calculating an estimate of daily dietary intake of VNT1, based on 95th percentile consumption rates and comparing this to daily protein intake.

For these calculations, the average weight of North American adults was 80.7 kg (Walpole et al., 2012) and the average weight of children aged 1 to 2 years was 11.0 kg (Langtree, 2016).

To calculate a conservative (high-end) estimate of the proportion of VNT1 consumed in the diet relative to daily protein intake, the subgroup with the lowest recommended daily intake of protein was used (adults aged 18-70; 0.66 g/kg bw/d) (Institute of Medicine, 2011). Because some adults may not consume their recommended daily intake of protein, 50% of that value was used in the dietary exposure calculations (0.33 g/kg bw/d).

Table 25. Per Capita Potato Consumption from DEEM-FCID

Consumption Subgroup	95th Percentile Potato Consumption g/kg bw/d[1]
Total U.S. Population[2]	2.85
Hispanic	2.84
Non-Hispanic-White	2.84
Non-Hispanic-Black	2.78
Non-Hispanic-Other	3.09
Nursing Infants	1.53
Non-Nursing Infants	3.64
Female 13+ Pregnant	2.76
Children 1-6	5.70
Children 7-12	3.61
Male 13-19	2.86
Female 13-19/Non-Pregnant	2.74
Male 20+	2.54
Female 20+/Non-Pregnant	2.22
Seniors 55+	2.36
All Infants	2.85
Female 13-50	2.27
Children 1-2[3]	7.14
Children 3-5	5.09
Children 6-12	3.78
Youth 13-19	2.80
Adults 20-49	2.37
Adults 50-99	2.38
Female 13-49	2.29

[1]DEEM 4.02, version 10, acute analysis for potato.
[2]Green-highlighted cells indicate the 95th percentile potato consumption for the total U.S. population.
[3]Yellow-highlighted cells indicate the 95th percentile potato consumption for the sub-population with the highest exposure to potato. Green and yellow numbers were used to calculate the amount of VNT1 consumed by the total U.S. population and children aged 1-2, respectively, from W8 tubers.

Table 26. Dietary Exposure of Humans to VNT1 from W8, X17, or Y9

DEEM Population	Potato Consumption at 95th Percentile	Estimated Exposure to VNT1 in Tubers[1]	Percent of Daily Protein Consumed that is VNT1[2]
Total U.S. population	2.85 g/kg bw/d	0.000284 mg/kg bw/d	0.000086%
Children Ages 1-2	7.14 g/kg bw/d	0.000714 mg/kg bw/d	0.00021%

[1]Exposure to VNT1 = (Consumption at 95th percentile) × (0.1 µg VNT1/g tubers), based on a conservative estimate of < 100 ppb.
[2]Percent of daily protein consumed = (exposure to VNT1/ 330 mg/kg bw/d).

This dietary exposure assessment is based on a number of conservative assumptions, meaning that exposure is likely overestimated. The data are from the United States, where the per capita consumption of potatoes is higher than in Asia. The estimate uses values from 95th percentile consumers of potato and assumes 100% of the potatoes consumed in the diet are either W8, X17, or Y9. It also assumes that the proportion of protein consumed in the diet is 50% less than the recommended amount for the lowest subgroup, which results in an increased proportion of VNT1 consumed in the diet. Furthermore, it assumes there is no degradation of the VNT1 protein in the harsh conditions associated with cooking and processing. These estimates point to negligible exposure for risk assessment purposes.

R-proteins are expressed at very low concentrations in plants. For instance, expression of the late blight resistance protein, Rpi-blb1 is estimated to be as low as 18 ppt in leaves, leading to low exposure in humans (3.6 ng protein/year) (Bushey et al., 2014). Therefore, dietary exposure of R-proteins to humans will be negligible.

In W8, X17, and Y9, significantly lower levels of *Rpi-vnt1* mRNA were measured in tubers compared to leaves (Section B1). The pattern of gene expression being higher in leaves and lower in tubers is consistent with other work on R-gene expression in potatoes (Pel, 2010). In W8, X17, and Y9 tubers, the VNT1 protein was not detectable by western blotting methods capable of detecting VNT1 at levels as low as 30 ppb in tubers. A conservative estimate of VNT1 in tubers was established to be less than 100 ppb. This conservative (upper-bound) estimate of VNT1 in W8, X17, and Y9 tubers was compared to 95th percentile potato consumption rates to calculate the dietary exposure of VNT1 for humans in the United States.

The exposure estimate for VNT1 was 0.000284 mg/kg bw/d for the total United States population, and 0.000714 mg/kg bw/d for children aged 1-2 (Table 26). In addition, the percentage VNT1 consumed compared to total protein intake is extremely low (0.000086% for the total United States population and 0.00021% for children aged 1-2).

These estimates indicate that dietary exposure to VNT1 from W8, X17, and Y9 will be negligible.

Dietary Exposure of Livestock to VNT1

The potential exposure of livestock to VNT1 from consuming W8, X17, or Y9 potatoes was evaluated by calculating an estimate of daily dietary intake of VNT1 for cows and pigs and comparing this to daily protein intake.

Potatoes are seldom fed to cows at high inclusion levels in diets due to operational and ration-balancing constraints (Charmley et al., 2006). Cows in the vicinity of potato processing plants are most likely to incur sustained exposure to potatoes in their diets. For example, Simplot cattle near processing plants in the Northwest United States are fed diets during the finishing phase that include up to 30% potato. These cattle eat approximately 18.1 kg of feed per day, which would include 5.4 kg fresh weight potato per day during the time when their diets include potatoes. Charmley et al., 2006 recommended a lower diet incorporation rate—20%—for potato fed to livestock, because increasing the level of potato in the diet beyond roughly 20% resulted in a decline in dry matter intake. The amount of protein in cattle feed varies depending on the diet and growth stage. An estimate of 12% protein in cattle feed on a dry matter basis, was used in the dietary exposure assessment.

Using this information with the higher 30% diet incorporation rate, the exposure estimate for VNT1 consumed by cattle was 0.00135 mg/kg bw/d (Table 27). The estimate conservatively assumes that 100% of the potato in a cow's diet is from W8 potatoes. When comparing the potential exposure of cattle to VNT1 with the amount of total daily dietary protein, the percentage VNT1 that cattle consume is negligible (0.000049%).

Table 27. Dietary Exposure Assessment of VNT1 for Livestock

Livestock	Average Finishing Weight	Potato Consumption[1]	Daily Protein Consumption (g/kg bw/d)[2,3]	Exposure to VNT1 (mg/kg bw/d)[4]	Percent of Daily Protein Consumed that is VNT1[5]
Cattle	400 kg	5.45 kg	2.721	0.00135	0.000049%
Pig	62 kg	0.9 kg	7.68	0.00144	0.000018%

[1]Potato consumption is based on a 30% incorporation rate.
[2]Cattle daily protein consumption = {[(9.05 × 0.12) × 1000 g/kg] / 400 kg}. Cattles feed is typically 50% dry matter.
[3]Pig daily protein consumption = {[(3 kg × 0.16) × 1000 g/kg] / 62.5 kg}.
[4]Exposure to VNT1 = (potato consumption × 0.1 µg VNT1/g tubers) / (average finishing weight). Based on a conservative estimate of < 100 ppb.
[5]Percent of daily protein consumed = (exposure to VNT1/ 1000 mg/g) / (daily protein consumption).

Sheep, as ruminants, may consume potatoes occasionally as a portion of their diet, but for a relatively short period of time. Research did not reveal any intentional, regular feeding of potatoes at sustained or high levels to sheep. Therefore, sheep exposure to VNT1 from consuming W8, X17, or Y9 potatoes would be substantially less than the negligible exposure for cows.

Pigs are fed cooked potatoes, as cooking prevents disruption of digestion by protease inhibitors (Charmley et al., 2006). Potato peel can be included at a level up to 30% in feed during the growing-finishing phase (van Lunen et al., 1989). Pigs weigh an average of 62.5 kg during their growing-finishing phase, based on a starting weight of 23 kg and an ending weight of 102 kg (van Lunen et al., 1989). For this estimate, a pig is assumed to consume 3 kg of feed per day (Table 7 of van Lunen et al., 1989) with 30% potato in the diet. Pig feed typically has a protein content of 16% on a dry matter basis (van Lunen et al., 1989). The exposure estimate for pigs consuming VNT1 in potatoes was 0.00144 mg/kg bw/d (Table 27). Furthermore, by comparing the potential exposure of pigs to VNT1 with the amount of total daily dietary protein intake (van Lunen et al., 1989), the percentage of VNT1 relative to total protein consumed by pigs is very low (0.000018%).

Like the cattle estimates, the exposure estimate calculated for pigs is based on conservative assumptions, including:

- 100% of the potato in a pig's diet is from W8, X17, or Y9 potatoes;
- A conservative estimate of <100 ppb of VNT1 in tubers; and
- No degradation of VNT1 occurs during processing or cooking of the potatoes.

Because the negligible exposure estimate rests on conservative assumptions, the actual exposure may be even lower.

Furthermore, the scientific literature reports the degradation of proteins during processing and digestion, as well as the existence of physical and biological barriers that further reduce potential exposure by restricting the movement of intact proteins into cells after consumption (Hammond et al., 2013). Proteins, unlike low-molecular weight chemicals, are large macromolecules composed of amino acids, which are broken down by digestive enzymes to support nutritional needs. When considering risk assessment, these facts underline the negligible potential exposure.

In summary, the estimated exposure of humans and livestock consuming W8, X17, or Y9 potatoes containing the VNT1 protein is negligible due to protein expression below the LOQ. Based on data from DEEM-FCID database, calculations using the 95th percentile of United States potato consumption showed that human consumption of VNT1 protein from W8, X17, or Y9 potatoes would be very low relative to daily protein intake. Based on the maximum recommended incorporation rates, the consumption of VNT1

protein from W8, X17, or Y9 potatoes would be a very small percentage of total protein consumed in livestock diets, even if these potatoes were to make up 100% of the potato products consumed. Therefore, the exposure of humans and livestock to VNT1 from W8, X17, or Y9 is negligible.

Conclusions of the Safety Assessment of VNT1

The safety of the VNT1 protein in W8, X17, and Y9 potatoes was evaluated using a weight-of-evidence approach that considered all VNT1 safety data and applied risk assessment principles (potential hazard X potential exposure = potential risk) to evaluate risk associated with consumption of VNT1.

The weight-of-evidence strongly supports VNT1 safety:

- The *Rpi-vnt1* gene is identical to the *Rpi-phu1* gene found in *S. phureja* potatoes, which has an established history of safe use. Therefore, the introduction of *Rpi-vnt1* into commercially grown potatoes should result in varieties with substantially similar food safety profiles to varieties with *Rpi-phu1*;
- The prevalence of R-genes similar to VNT1 in edible crops suggests that R-proteins are widespread in nature, and the VNT1 protein is similar to proteins already present in the food supply with a history of safe consumption;
- The biological mechanism of disease protection by VNT1 is the triggering of an existing hypersensitivity response in the plant, which is conserved among plants containing R-genes. This supports the safe use of VNT1 to introduce disease protection into edible crops;
- Bioinformatic analysis confirms that VNT1 lacks sequence similarity to known toxins and allergens;
- Homology of VNT1 to other proteins in tomato, pepper, and potato with a history of safe use provides additional evidence that VNT1 in W8, X17, and Y9 potatoes is as safe for human consumption as R-proteins similar to VNT1 in other foods; and
- The potential exposure for humans and livestock to VNT1 is negligible.

Based on data from the DEEM-FCID database, calculations based on the 95th percentile of United States potato consumption demonstrated that human consumption of VNT1 protein from W8, X17, and Y9 potatoes would be a very small percentage of total protein consumed in the diet, even if W8, X17, and Y9 potatoes comprised 100% of potato products consumed.

Based on maximum recommended incorporation rates, the consumption of VNT1 protein from W8, X17, and Y9 potatoes would be a very small percentage of total protein consumed in livestock diets, even if W8, X17, and Y9 potatoes were 100% of potato products consumed.

When both the minimal hazard and the negligible exposure potential are considered together, the potential risk associated with consuming VNT1 from W8, X17, and Y9 potatoes is close to zero. The VNT1 protein in W8, X17, and Y9 potatoes is as safe for humans, livestock, and other consumers as similar R-proteins in commonly consumed foods.

B.1(c) information on whether any new protein has undergone any unexpected post-translational modification in the new host

The VNT1 protein is expressed from a potato gene, *Rpi-vnt1* that is native to a related species, *S. venturii*. The cassette that was used to introduce this gene into Russet Burbank, Ranger Russet, and Atlantic varieties, included the native promoter and terminator sequences from the original host, *S. venturii*. The transformed varieties show efficacy against *P. infestans* and expression studies showed similar levels of expression of the gene in foliar tissue when compared to *S. venturii*, suggesting that the gene and protein are processed similarly. Post-translational modifications (PTMs) to VNT1 cannot be evaluated as expression levels are below the limit of detection, but differences in PTMs due to cellular processing enzymes are unlikely given that the original and new hosts are closely related. Thus, differences in PTMs of VNT1 between the hosts are not expected.

B.1(d) where any ORFs have been identified (in subparagraph A.3(c)(v) of this Guideline (3.5.1)), bioinformatics analyses to indicate the potential for allergenicity and toxicity of the ORFs

The sequences of the genetic elements in the pSIM1278 and pSIM1678 cassettes are derived from Ranger Russet conventional potatoes with the exception of *Rpi-vnt1*, which is from the wild species *Solanum venturii* (Foster et al., 2009). Expression of allergens or toxins is unlikely, particularly in potatoes transformed with potato DNA.

An analysis was completed using bioinformatic techniques (Goodman et al., 2008; Ladics et al., 2007; Terrat and Ducancel, 2013) to determine homology between known toxins or allergens and open reading frames (ORFs) introduced into events W8, X17, and Y9 through transformation with pSIM1278 and pSIM1678 (Report16-47-SPS-MOL, Report16-48-SPS-MOL and Report16-49-SPS-MOL). A summary of the methods used to identify ORF sequences and evaluate the sequences against known allergens or toxins is provided in Table 28. Most of the ORFs contained in the pSIM1278 and pSIM1678 inserts already exist as part of the potato genome, as the inserts are derived from potato DNA.

Table 28. Overview of Analyses Using Bioinformatics

Analysis	Purpose	Approach
Start-to-stop ORF Analysis	Identify all open reading frames associated with the pSIM1278 and pSIM1678 inserts, including junction regions.	Python script: systematically identify all ORFs (≥30 amino acids) located between a start codon and a stop codon where all six reading frames are considered.
Allergenicity Analysis	Confirm that known allergenic sequences have not been introduced through transformation.	AllergenOnline (FASTA Search): identify any small regions of identity or larger regions of homology between ORFs and known allergens.
Toxicity Analysis	Confirm that sequences similar to known toxins have not been introduced through transformation.	BLAST (blastp) search: identify any ORFs with homology to proteins with “toxin” in its NCBI annotation.

Identification of ORFs Associated with pSIM1278 and pSIM1678

All allergen and toxin assessments were performed using the following sequences:

- The VNT1 protein sequence (891 amino acids; ACJ66594) expressed from *Rpi-vnt1* in pSIM1678 and
- All other ORFs, including junction regions, associated with the pSIM1278 and pSIM1678 inserts.

A start-to-stop ORF was defined as the contiguous sequence between an AUG start codon and the subsequent in-frame stop codon. Since a nucleotide sequence can be translated in three reading frames from two directions, all six reading frames of the pSIM1278 and pSIM1678 inserts and flanking regions were analysed for ORFs (shown schematically in Figure 52). The results were converted into FASTA-formatted files, using CLC Genomic Workbench software (Qiagen). All start-to-stop ORFs, at least 30 amino acids, contained within the T-DNA insert, including the VNT1 protein sequence and those adjacent to flanking genomic sequence (junction ORFs), were identified and used in the subsequent allergen and toxin analyses.

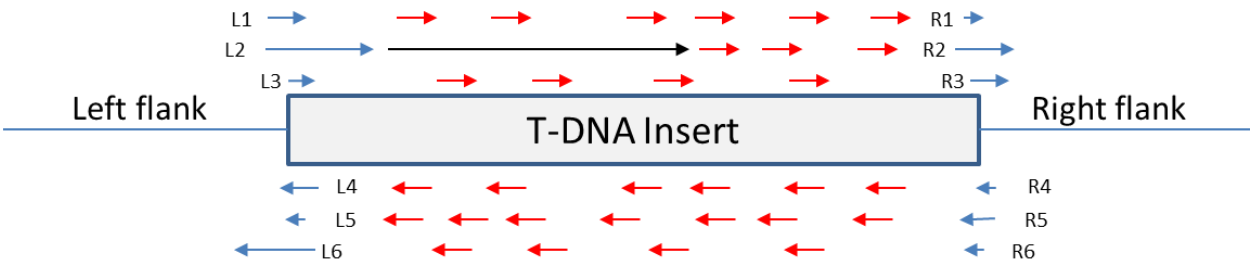


Figure 52. Complete ORF Analysis Scheme

A schematic diagram illustrating a representative T-DNA insertion site in the plant genome. All ORFs (≥30 amino acids) contained within the DNA insert (red lines), including an introduced protein (e.g. *Rpi-vnt1*) coding region (black line), or overlapping the junctions between the insert and the plant genome (blue lines) are identified for each insert and used in subsequent analyses. All lines are representative and do not indicate actual ORFs.

Allergenicity Searches

A series of searches was conducted to identify matches between the protein sequence query and known allergens compiled in the 2016 AllergenOnline.org database, available through the Food Allergy Research and Resource Program (FARRP) via the University of Nebraska (http://www.allergenonline.org/databasefasta.shtml). The AllergenOnline database is a peer-reviewed database that is updated annually. Deposited sequences in the database are subject to review for appropriateness by a panel of qualified food allergenicity experts. Version 16, utilised for the present search contains 1,956 deposited sequences, and was searched using FASTA to identify sequence identity between input and database entries (Pearson and Lipman, 1988). Search methods are supported by recent published guidance for protein allergenicity prediction in food products, and are based on sequence identity as well as structural aspects of the interaction between antibodies and protein targets (cross-reactivity potential) (Goodman et al., 2008; Ladics, 2008; Ladics and Selgrade, 2009).

A match between the input protein sequences and sequences of known or suspected allergens catalogued in the database indicates theoretical potential for cross-reactivity between a protein containing the query sequence and existing antibodies to food allergens present in allergic individuals. Antibodies present in the serum of allergic individuals recognise specific antigens, and upon recognition, elicit a signalling cascade that induces allergic responses. Significant identity between the queried sequence and the known or

suspected allergens in the database indicates that there could be potential for the queried protein to elicit an allergic response in individuals with allergies to the identified protein.

Full-Length Sequence Search

This database search used FASTA alignment to identify matches between full-length sequence queries and known or putative allergens contained in the AllergenOnline database. Search results with significant homology were determined using two general criteria: (1) greater than 50% identity between the query protein and database entry, and (2) an E-value less than 10^{-4}. The E-value describes the number of hits one can expect to see by chance when querying a database of a particular size. The E-value effectively describes random background noise that exists for matches between sequences in a particular database. Lower E-values (e.g. $<10^{-4}$) indicate proteins have increased similarity to the target and are less likely to be due to chance.

80-mer Sliding Window Search

The 80-mer sliding window search identifies localised regions of similarity between the ORFs and known allergens by comparing all contiguous 80 amino acid sequences within an ORF to sequences in the AllergenOnline database. Matches were defined as sequences having greater than 35% homology to known allergens (E-value cutoff = 10^{-4}). The premise for this search is the variability of antibodies that allows recognition of specific proteins and is used to determine the potential of an ORF to interact with an antibody and cause a response. Sequences in the database with less than 35% identity are not considered likely candidates for cross-reactivity (Ladics, 2008; Ladics and Selgrade, 2009). Such proteins are therefore not detailed in the search output from the database.

8-mer Exact Match Search

The 8-mer exact match search identifies regions of 8 amino acid identity between the queried ORF sequence and known or suspected allergens in the database. There is no scientific rationale for considering very small regions of identity (i.e. 6-8 amino acids) a safety concern, unless substantiated by other search methods (Goodman et al., 2008). Although the frequency and occurrence of matching 8-mer sequences is used to assess the potential for cross-reactivity, FARRP warns that the 8-mer search can lead to identification of false positive results, and suggests caution should be used when interpreting findings based on 8-mer matches between the queried sequence and known or putative allergens.

Toxicity Searches

Methods familiar to regulators and established in some countries include an approach modelled after the allergenicity studies where bioinformatics is used to inform on the potential for sequence similarity between protein sequences and known toxins.

The NCBI database was queried using all protein sequences annotated with the keyword “toxin” (Entrez query: “toxin”; E-value < 10^{-2}). All matches are reported. For those matches subsequently determined not relevant (i.e., false positives), a brief explanation and rationale for safety is included. Protein sequences evaluated using this method may include actual toxins, proteins involved in the synthesis of toxins in the host, proteins that interact with the toxins, proteins involved in the defence or response to infection, or non-toxic proteins from organisms known to produce a toxin.

Allergen and Toxin Homology Assessment of VNT1 in Events W8, X17, and Y9

The sequence of the VNT1 protein was evaluated for allergenicity and toxicity potential and was found unlikely to pose a risk as either an allergen or toxin.

Assessment of VNT1 Allergen Homology

A FASTA search using the full-length VNT1 protein did not identify any allergens that satisfied the search parameter threshold (E-value < 10^{-4}). The full-length protein has no significant sequence identity with known or suspected allergens.

An 80-mer sliding window search using the VNT1 protein sequence did not identify any allergens that satisfied the search parameter threshold (>35% identity over 80 amino acids, E-value < 10^{-4}). The VNT1 protein has no significant sequence identity with known or suspected allergens.

The 8-mer exact match search evaluated all possible 8-mers in VNT1 for identity with known or putative allergens in the database. The 8-mer search did not identify any matches with a potential allergen concern.

Assessment of VNT1 Toxin Homology

A BLAST (blastp) search performed against protein sequences in the comprehensive NCBI database was targeted to records annotated with the keyword "toxin". The search results did not identify any protein toxins, but did identify a number of proteins involved in the response to toxins that contain the keyword "toxin" in the record (Table 29). There are no known toxins with significant sequence identity to VNT1.

Table 29. Summary of Search Results for Toxin Potential of VNT1 in Events W8, X17 and Y9

Query Match	Accession
LOV1 (*Arabidopsis thaliana*): Confers susceptibility to the fungus *Cochliobolus victoriae* by conditioning victorin-dependent (victorin is a toxin synthesised by *C. victoriae*) induction of defense-associated proteins.	A7XGN8, A9QGV6
PREDICTED (*Erythranthe guttata*): Putative late blight resistance protein homolog R1A-3. Region 4-120: Toxin_10; note = "Insecticidal Crystal Toxin, P42; pfam05431".	XP_012846417
Hypothetical protein MIMGU_mgv1a017786mg, partial (*Erythranthe guttata*): Region 4-120: Toxin_10; note = "Insecticidal Crystal Toxin, P42; pfam05431".	EYU29791
RP3-like (*Sorghum bicolor*): rp3-like disease resistance protein in the Pc locus of *S. bicolor*, confers resistance to Pc toxin.	ACE86400, ACE86402, ACE86396
Tsn1 (*Aegilops speltoides*): S/T protein kinase-NBS-LRR-like protein, confers sensitivity to the wheat fungal pathogen ToxA.	ADG84875, ADG84876, ADG84877

Three of the matches identified, LOV1, RP3-like, and Tsn1, are R-protein homologs that function in the sensitivity of their host to fungal pathogens through recognition of effector molecules, i.e. victorin, Pc toxin, and ToxA. A literature review did not reveal these R-proteins to be toxins or have toxic properties (Faris et al., 2010; Lorang et al., 2007; Nagy and Bennetzen, 2008; Walton, 1996).

Matches between VNT1 and a predicted putative late blight resistance protein homolog (R1A-3) and a hypothetical protein, both from *Erythranthe guttata*, resulted from the search. *E. guttata* is an ornamental

flower not consumed by humans. The sequences of these proteins were annotated as containing a region of similarity with a protein family called Toxin_10. Toxin_10 is a family of insect-specific protein toxins, including Cry35, Cry36, BinA, and BinB from *Bacillus* species. Although this region is annotated as Toxin_10, the sequence identity between the *E. guttata* predicted proteins and other Toxin_10 annotated proteins is low (15-22%) and is unlikely to have toxic potential.

The allergen and toxin homology assessment of the VNT1 protein did not identify any safety concerns.

Allergen and Toxin Homology Assessment of pSIM1278 and pSIM1678 ORFs in W8, X17, and Y9

The sequences of all ORFs, including VNT1 (see above), contained within the pSIM1278 and pSIM1678 inserts or associated with the junctions or integration sites of the inserts, were evaluated for allergen or toxin homology. Neither the toxin assessment or the allergen assessment identified any safety concerns related to any of the ORFs identified in events W8, X17 and Y9. Full details are provided in the following reports:

- Report 16-47-SPS-MOL, W8;
- Report 16-48-SPS-MOL, X17;
- Report 16-49-SPS-MOL, Y9;

Assessment of ORF Allergen Homology

A full-length search was performed to identify homology between ORFs in the inserts and known allergens. The algorithm identified a match between an ORF within the pSIM1678 insert and an allergen characterised as a minor tomato allergen within the FARRP database (Table 30). The identity between the pSIM1678 ORF and the tomato invertase sequence was high (99%), but expected as the pSIM1678 insert includes an inverted repeat from the homologous vacuolar invertase (*VInv*) gene from potato. The inverted repeat leads to down regulation of the vacuolar invertase in potato.

An 80-mer sliding window search using the ORF sequences confirmed homology with the minor tomato allergen.

Table 30. Summary of Allergen Homology of pSIM1278 and pSIM1678 ORFs in W8, X17 and Y9

Query Match	Accession
Minor allergen beta-fructofuranosidase precursor – vacuolar invertase (*Solanum lycopersicum*) (Foetisch et al., 2003)	AAL75449, AAL75450

It is not surprising that an ORF including the VInv sequence from the pSIM1678 T-DNA would align to the vacuolar invertase protein from tomato, as the full-length proteins are 95% identical (potato: PGSC0003DMP400024451, Michigan State University Spud DB; tomato: accession AAL75449, GenBank). However, this ORF is not considered a safety concern for the following reasons:

- The amino acid sequence predicted from the ORF associated with the *VInv* fragment in pSIM1678 exists naturally in potatoes as part of the potato vacuolar invertase protein. The *VInv* region of the pSIM1678 T-DNA was developed using *S. tuberosum* var. Ranger Russet sequence. Therefore, individuals sensitive to the tomato vacuolar invertase protein are no more likely to experience an allergic reaction with W8, X17, or Y9 than with conventional potatoes;

- Translation of an ORF associated with the VInv cassette in pSIM1678 is unlikely, as the transcript containing the inverted repeat sequences forms dsRNA that is processed by the RNAi pathway into small interfering RNA (i.e. siRNA), which prevents the transcript from serving as a template for ribosomal translation. This means the *VInv* ORF is unlikely to lead to a novel protein in W8, X17, or Y9, further reducing allergenicity concerns associated with this ORF; and
- The VInv cassette leads to production of siRNA that down-regulate the potato vacuolar invertase gene. Similarly, these siRNAs would target any other transcripts for degradation containing the *VInv* sequence, including any transcripts from the ORF spanning the VInv cassette, lowering production of any potentially allergenic endogenous proteins. Thus, the VInv cassette reduces the amount of vacuolar invertase in W8, X17, and Y9 compared to conventional potatoes, further reducing allergenicity concerns associated with this ORF.

The 8-mer exact match search of pSIM1278 and pSIM1678 ORFs did not identify any matches that would indicate a potential safety concern. The search results confirmed the high identity (99%) between the ORF in the pSIM1678 insert and the tomato vacuolar invertase sequence (Table 30).

No other matches were identified from the full-length, 80-mer, or 8-mer searches. The ORFs associated with the pSIM1278 and pSIM1678 inserts are unlikely to pose an allergen-related safety concern.

Assessment of ORF Toxin Homology

A BLAST (blastp) search of the ORFs associated with the pSIM1278 and pSIM1678 inserts did not identify any annotated toxins. Matches to the VNT1 protein were presented above (Table 29). A number of proteins were identified that are homologous to the partial invertase (*VInv*) sequence in the pSIM1678 construct. The matches are not themselves toxins, but represent a number of sucrose degrading enzymes ubiquitously expressed in bacteria (

Table 31). They do not pose a risk to humans or other organisms.

Allergen and Toxin Homology Assessment of Junction ORFs

The sequences of the ORFs covering the junctions between the inserts and the potato flanking regions were evaluated as part of the ORF analysis. Details of ORFs associated with the junction regions of events W8, X17, and Y9 are presented in Section A.3(c)(v) provided in SUPPLEMENT 1. The analysis did not identify any safety concerns.

Table 31. Summary of Search Results for Toxin Potential of ORFs in W8, X17 and Y9 associated with pSIM1278 and pSIM1678

ORFs from pSIM1278

Query Match	Source Organism	Accession
Asparagine Synthetase B	*Escherichia coli*	AIZ81697, KFD76705, NP_308731, EIL13740, EYZ18706, EYY50888, EZH10307, EIL02389, EYY57333, EIL08426, KDV14966, EYU77209, EIL30960, EZA17347, EYV14508, KNZ12093, EYW20264, EZA37061, EZB27777, EYV89799, EYZ96359, EJF05567, ANO76830
Asparagine Synthetase	*Vibrio cholerae*	EEY43083
Asparagine Synthase	*Clostridium botulinum*	KIL09560

ORFs from pSIM1678

Query Match	Source Organism	Accession
Glycosyl Hydrolase Family 32, Sucrose-6-Phosphate Hydrolase	*Escherichia coli*	KOZ65786, NP_311270, EYW80343, EYZ93717, EYV91737, KOZ09537, EZB27384, EYZ68862, EDV64124, EYY18841, EYZ04892, KDV15364, KFD75665, EZQ28926, EYV93762
Sucrose-6-Phosphate Hydrolase, Beta-Fructofuranosidase	*Enterococcus faecalis*	EFK77719, ELA02140
Sucrose-6-Phosphate Hydrolase, Beta-Fructofuranosidase	*Bacillus cereus*	BAL16531, EDX61098
Sucrose-6-Phosphate Hydrolase	*Vibrio cholera*	EEY52113, EEY41740, EEY48061
Sucrose-6-Phosphate Hydrolase	*Enterococcus faecium*	EFF60448, EFF59908, EFF60441
Sucrose-6-Phosphate Hydrolase	*Clostridium botulinum*	KIL08234
Sucrose-6-Phosphate Hydrolase	*Staphylococcus hyicus*	AJC95603
Sucrose-6-Phosphate Hydrolase	*Staphylococcus aureus*	KPE20402, KPE18748
Hypothetical protein	*Ensifer sp.*	WP_026617525

B.2. New Proteins

B.2 (a) and (b) Information on potential toxicity and allergenicity

The pSIM1678 T-DNA contains the *Rpi-vnt1* gene found in *S. venturii* and *S. phureja.* The gene is expressed under the native *Rpi-vnt1* promoter and terminator. A detailed description of the history and mode of action of VNT1 can be found in Section B.1(b).

Details of the potential toxicity and allergenicity of the protein VNT1 are presented in the following Sections:

- Section A.2(a)(i) and
- Section B.1(d)

VNT1 Protein Identity

The *Rpi-vnt1* gene (accession: FJ423044) is one of three isoforms identified in the wild species *S. venturii*. The *Rpi-vnt1* gene sequence is identical to the *Rpi-phu1* gene from the related species, *S. phureja,* and a homolog of the Tm-2^2 tomato mosaic virus (ToMV) disease resistance gene in tomato (Foster et al., 2009; Śliwka et al., 2013). The gene encodes the 891 amino acid R-protein designated VNT1 (Figure 53).

The recognition of pathogen-secreted effectors (e.g. Avr-vnt1) by R-proteins is one of the most studied mechanisms in plant defense response (Panstruga et al., 2009). R-proteins such as VNT1 are signal transduction ATPases with homologs found in all domains of life (Leipe et al., 2004). Most known disease resistance R-proteins contain a nucleotide-binding site (NBS or NB) and leucine-rich repeat (LRR) domain (Lozano et al., 2012). Two classes of R-proteins with distinct motifs have been identified that contain the NBS/NB-LRR domains:

- N-terminal toll/interleukin 1 receptor (TIR)(TIR-NB-LRR)
- N-terminal coiled-coil (CC-NB-LRR).

The *Rpi-Vnt1* gene and other R-genes that provide protection against *P. infestans* (known as *Rpi* genes) typically encode immune receptor proteins of the coiled coil, nucleotide binding, leucine rich repeat (CC-NB-LRR) class of intracellular plant proteins (Vleeshouwers et al., 2011).

```
001 MNYCVYKTWA VDSYFPFLIL TFRKKKFNEK LKEMAEILLT AVINKSIEIA
051 GNVLFQEGTR LYWLKEDIDW LQREMRHIRS YVDNAKAKEV GGDSRVKNLL
101 KDIQQLAGDV EDLLDEFLPK IQQSNKFICC LKTVSFADEF AMEIEKIKRR
151 VADIDRVRTT YSITDTSNNN DDCIPLDRRR LFLHADETEV IGLEDDFNTL
201 QAKLLDHDLP YGVVSIVGMP GLGKTTLAKK LYRHVCHQFE CSGLVYVSQQ
251 PRAGEILHDI AKQVGLTEEE RKENLENNLR SLLKIKRYVI LLDDIWDVEI
301 WDDLKLVLPE CDSKIGSRII ITSRNSNVGR YIGGDFSIHV LQPLDSEKSF
351 ELFTKKIFNF VNDNWANASP DLVNIGRCIV ERCGGIPLAI VVTAGMLRAR
401 GRTEHAWNRV LESMAHKIQD GCGKVLALSY NDLPIALRPC FLYFGLYPED
451 HEIRAFDLTN MWIAEKLIVV NTGNGREAES LADDVLNDLV SRNLIQVAKR
501 TYDGRISSCR IHDLLHSLCV DLAKESNFFH TEHNAFGDPS NVARVRRITF
551 YSDDNAMNEF FHLNPKPMKL RSLFCFTKDR CIFSQMAHLN FKLLQVLVVV
601 MSQKGYQHVT FPKKIGNMSC LRYVRLEGAI RVKLPNSIVK LKCLETLDIF
651 HSSSKLPFGV WESKILRHLC YTEECYCVSF ASPFCRIMPP NNLQTLMWVD
701 DKFCEPRLLH RLINLRTLCI MDVSGSTIKI LSALSPVPRA LEVLKLRFFK
751 NTSEQINLSS HPNIVELGLV GFSAMLLNIE AFPPNLVKLN LVGLMVDGHL
801 LAVLKKLPKL RILILLWCRH DAEKMDLSGD SFPQLEVLYI EDAQGLSEVT
851 CMDDMSMPKL KKLFLVQGPN ISPISLRVSE RLAKLRISQV L
```

Figure 53. Amino Acid Sequence of VNT1

The *Rpi-vnt1* gene encodes an 891 amino acid R-protein (102 Kda). Individual domains are highlighted, coiled-coil (CC)

(green), nucleotide binding (NBS) (blue), and leucine rich repeat (LRR) (brown).

B.3. Other (non-protein) new substances

If other (non-protein) substances are produced as a result of the introduced DNA, information must be provided on the following:

B.3(a) the identity and biological function of the substance

B.3(b) whether the substance has previously been safely consumed in food

B.3(c) potential dietary exposure to the substance

Transcription of the inverted repeats leads to down regulation of asparagine synthetase, polyphenol oxidase, phosphorylase L, water dikinase, and vacuolar invertase through production of dsRNA and the plant's RNAi pathway. The inverted repeats are derived from the DNA sequences from five potato genes (*Asn1*, *Ppo5*, *PhL*, *R1,* and *VInv*; Table 2).

The T-DNA in each plasmid contain down-regulation cassettes, that result in the production of siRNA in W8, X17, and Y9 using the plant's RNAi pathway. As described in Section A.3(b), each down-regulation cassette is comprised of DNA sequence arranged as an inverted repeat.

Due to the nature of the inverted repeat sequences, their transcripts form dsRNA through complementary binding. The dsRNA act as a precursor for the plant's own RNAi post-transcriptional regulatory pathway. A cellular RNase III enzyme, Dicer, recognises and processes the precursor dsRNA into 21-24 bp duplexes termed siRNA. The siRNA bind with cellular proteins forming RNA Induced Silencing Complexes (RISC). The RISC selectively degrades one of the siRNA strands, referred to as the passenger strand. The remaining strand, referred to as the guide strand is used to target the complementary sequence in mRNA molecules. Once the guide strand pairs with an mRNA in a RISC complex, the mRNA molecule is cleaved and degraded, preventing protein translation (Chau and Lee, 2007; Fire et al., 1998).

FSANZ are currently assessing potato event E12 (A1128), transformed with pSIM1278. At the time of submission, FSANZ had not identified any potential public health and safety concerns.

Safety Assessment of Small RNA Generated in W8, X17, and Y9 Potatoes

Reduced black spot, reduced free asparagine, and lower reducing sugars were achieved using RNAi to down regulate asparagine synthetase, polyphenol oxidase, phosphorylase L, water dikinase, and vacuolar invertase in W8, X17, and Y9. This resulted in reduced levels of mRNA transcripts for these enzymes.

A weight-of-the-evidence approach was used to support the safe consumption of small RNA in W8 potatoes. The analysis included:

- The history of safe use of dsRNA and siRNA in food and feed;
- A summary of the many biological barriers that limit the uptake and activity of small RNA in mammalian cells;
- A bioinformatic analysis comparing complementarity of potential siRNAs from W8, X17, and Y9 inserts to transcripts in humans and livestock commonly fed potatoes; and
- A conservative dietary exposure assessment for humans and livestock to siRNA in W8, X17, and Y9 potatoes.

History of safe use of dsRNA and siRNA

RNAi refers to a cellular pathway used by plant and animal cells to down regulate gene expression through degradation of targeted mRNA within the cell. Cellular enzymes detect long dsRNA and process them into small (21-24 nucleotide) interfering RNA (siRNA) (Hammond, 2005). The resulting siRNA pair with cellular

proteins which use one strand of the siRNA sequence to bind complementary mRNA sequences. Once bound by the siRNA-protein complex, the targeted mRNA sequence is cleaved, preventing protein translation (Chau and Lee, 2007; Fire et al., 1998).

The use of siRNAs to regulate gene expression through processing of endogenous dsRNA is common and highly conserved in plants, insects, fungi, nematodes, and animals. Traditional breeding practices have resulted in a number of conventional cultivars that produce siRNA and dsRNA that down regulate genes (Parrott et al., 2010; Petrick et al., 2013). Soybean seed colour, maize stalk colour, and rice protein content (Della-Vedova et al., 2005; Kusaba et al., 2003; Tuteja et al., 2004) are all examples of traits obtained through conventional breeding that utilize endogenous siRNA and dsRNA to regulate gene expression.

Published scientific literature demonstrates that consumption of biotechnology-derived crops using RNA-based gene regulation, such as siRNA, microRNA (miRNA), or dsRNA, is as safe for humans and livestock as other crops using RNAi technology (Parrott et al., 2010; Petrick et al., 2013). Since 1994, over thirty RNAi based biotech events have been approved globally for food, feed or cultivation in crops including alfalfa, apple, bean, potato, squash, plum, papaya, soybean, and tomato. Together, these events make up over 130 food and feed approvals in sixteen countries and have been consumed safely by humans and animals (ISAAA, 2015).

RNA transcripts, such as dsRNA and siRNA, are composed of nucleotides which are the basic units of both DNA and RNA. They are regularly consumed as part of the diet and are considered safe:

- In 2001, the United States EPA established an exemption from the requirement for a tolerance for residues of nucleic acids (40 C.F.R. 174.507) under the Federal Food, Drug, and Cosmetic Act (FFDCA), noting that “nucleic acids are ubiquitous in all forms of life, have always been present in human and domestic animal food and are not known to cause any adverse health effects when consumed as part of food” (66 Fed. Reg. 37817, July 19, 2001);
- The United States FDA reached a similar conclusion, stating that nucleic acids are “generally recognised as safe” (57 Fed Reg. 22984, 22990, May 29, 1992). “Introduced nucleic acids, in and of themselves, do not raise safety concerns. Thus, for example, the introduction of a gene encoding an anti-sense ribonucleic acid (RNA) would not raise concerns about either the gene or the anti-sense RNA. Any safety considerations would focus on the intended effects of the anti-sense RNA” (FDA, 1992); and
- In 2013, the bi-national government agency, Food Standards Australia New Zealand, which evaluates food safety requirements from biotech foods stated, “There is no scientific basis for suggesting that small dsRNA present in some biotech foods have different properties or pose a greater risk than those already naturally abundant in conventional foods” (FSANZ, 2013).

In alignment with the history of international approvals, there is regulatory consensus on the history of safe consumption of RNA including RNA transcripts, such as dsRNA and siRNA.

Plant tissues have several mechanisms to produce long dsRNA. Analysis of plant transcriptomes suggests that dsRNA are abundant in plants, with over 8 million long dsRNA predicted in conventional corn, soy, rice, lettuce, and tomato crops (Jensen et al., 2013). Many of these naturally abundant dsRNA have perfect complementarity to human genes and transcripts (Jensen et al., 2013). For example, a large number of small RNA in conventional rice grains have 100% complementary to nucleic acid sequences in the genomes and transcriptomes of mammals, including humans (Ivashuta et al., 2009).

The abundance of small RNA in food and their complementarity to animal genomes and transcriptomes provides strong evidence for a history of safe consumption, which is confirmed by the safe use of RNAi in approved biotechnology-derived crops.

Biological Barriers to siRNA Uptake

Many studies have examined the stability of small RNA and the biological barriers that limit uptake and activity of small RNA in mammals. Much of this research was aimed at optimising RNA for increased stability and improved uptake so that it could be used in therapeutics. RNA is extremely labile. Unlike DNA, RNA contains ribose with a hydroxyl group attached to the pentose ring in the 2' position. This hydroxyl group makes RNA less stable than DNA because it is more prone to hydrolysis. In addition, RNA is very susceptible to oxidation, hydrolytic cleavage by metallic complexes, and degradation by RNase enzymes (Fabre et al., 2014).

For humans and livestock that eat cooked potatoes, high temperature processing treatments (e.g., boiling, baking, frying) shut down cell metabolism and halt the replenishment of RNA. Changes in temperature and physical shearing associated with food processing and preparation degrades RNA leading to loss of activity. Exposure to siRNA is also reduced by numerous biological barriers that limit RNA uptake and activity in mammalian cells.

A summary of biological barriers to RNA uptake from food and examples of associated studies include:

- Degradation in saliva during chewing
    - 62% loss of activity was observed after exposure to saliva for 10 minutes (Hickerson et al., 2008);
- Degradation in the gastro-intestinal (GI) tract and stomach
    - Approximately 95% of plant-derived miRNA in the GI tract and stomach were eliminated within 2 hours and this increased to 99.6% after 24 hours (Liang et al., 2014);
    - The level of maternal milk-derived miRNA found in the intestines of sucklings is three orders of magnitude lower than in the stomach following suckling (Title et al., 2015);
- Degradation in the rumen
    - Free RNA fed to sheep and cows was rapidly degraded in the rumen to oligonucleotides, nucleosides and bases (McAllan, 1982);
- Inhibition of absorption from the GI tract to the plasma
    - The charged polyanionic structure and relatively large size of siRNA restrict absorption (Witwer and Hirschi, 2014)
    - Absorption of plant miRNA delivered by feeding was measured in human plasma and was detectable over a range from 0 - 1.31% (Liang et al., 2015). Similar numbers were measured in mice (0.3-1.8%) when administered by oral gavage, which does not include salivary-based degradation (Liang et al., 2014)
    - Low levels in plasma were measured following oral gavage (0.3%) or direct intestinal injections (0.9-2.4%) of synthetic, non-hydrolysable, oligonucleotides (Nicklin et al., 1998; Raoof et al., 2004)
    - Small therapeutic oligonucleotides formulated to optimise stability and absorption have only managed to modestly increase this number to 9.5% (Tillman et al., 2008)
    - There was no evidence for uptake of maternal milk-derived miRNA in suckling intestinal epithelium, gastric epithelium, plasma, liver, or spleen cells (Title et al., 2015);
- Degradation in the plasma

  - siRNA duplexes incubated in mouse plasma were rapidly degraded with 100% degradation recorded within 10 minutes. The siRNA were shown to be hydrolyzed to nucleosides in this process (Christensen et al., 2013)
  - A time course analysis was performed on the small number of plant-derived miRNA that were detectable in the plasma after administration to mice through ingestion. Most of the levels peaked at 6 hours and were undetectable within 9 hours of ingestion (Liang et al., 2014)
  - RNA molecules injected intravenously in mouse studies are rapidly cleared from the plasma by renal filtration and excretion (Petrick et al., 2013);

- Prevention of uptake from plasma into animal cells
  - The charged polyanionic structure and relatively large size of siRNA restrict absorption (Witwer and Hirschi, 2014) so that even tissue culture uptake requires the use of enhancing agents, e.g., lipofectamine
  - Challenges associated with inefficient cellular uptake have hindered the use of small RNA as therapeutic agents (Singh et al., 2011);
- Cellular barriers to biological activity
  - Biological activity in the cell requires that small RNA escape endosomes to prevent their degradation (Pei et al., 2010)
  - Small RNA must be loaded onto a limited number of Argonaut proteins in the cell in order to serve as a guide strand for either miRNA or siRNA-based gene regulation (Wang et al., 2010)
  - Studies performed to evaluate the concentration requirements for small RNA have shown that 370 to 18,000 copies/cell of a small RNA were required to silence a target RNA by 50% to 87% when using non-hydrolysable small RNA and liposome-mediated transfection (Pei et al., 2010). This level of absorbed siRNA is unlikely based on the barriers to uptake and the absence of an siRNA amplification mechanism in mammalian cells; and
- Scalable impact on mammalian cells
  - Amplification of siRNA in the host cell typically requires RNA-dependent RNA polymerase which is not found in vertebrates (Petrick et al., 2013; Witwer and Hirschi, 2014). Thus, humans or livestock would need to consume siRNA continuously and have an efficient uptake mechanism to maintain siRNA at levels that effectively down regulate genes in mammalian cells.

Collectively, these data indicate that small RNA derived from W8, X17, and Y9 potatoes will undergo degradation and encounter barriers that limit them from accumulating in mammalian cells at levels sufficient to affect gene expression. Generally, in order to affect an individual organism, complete complementarity between siRNA and an off-target mRNA must exist. Furthermore, many cells in one or more organs would need to be adversely affected. Biologically significant levels of plant siRNA have not been detected in mammals consuming plant material.

Bioinformatic Analysis of pSIM1278 and pSIM1678 Derived siRNA

Down regulation through RNAi requires complementarity between siRNA and mRNA in targeted cells (Jensen et al., 2013). To determine if sequence complementarity exists between siRNA in W8, X17, and Y9

and mRNA in humans, all the potential siRNA were queried against transcripts from NCBI's annotated RNA database (Pruitt et al., 2012).

RNA Database Searches

The NCBI database houses sequences for many organisms, but it can be queried (according to a taxonomy identifier) to target the search against specific organisms. A query using potato transcripts provided a positive control for the searches.

This query resulted in 1108 total matches between potential siRNA and potato transcripts, including the expected potato transcripts: Asparagine synthetase (XM_006343993.2), Polyphenol oxidase (XM_015304410.1), Phosphorylase L (NM_001288286.1), and Vacuolar Invertase (beta-fructosidase; NM_001288064.1).

A single transcript in the human database (XR_913817) was identified that matched siRNA from the PhL/R1 inverted repeat (Table 32). That Accession, XR_913817, is annotated in the NCBI database as an uncharacterised noncoding RNA (Table 33).

Table 32. NCBI RefSeq_RNA Database Summary by Organism and Inverted Repeat

Organism (Taxonomy ID)	Number of Sequences Compared	Asn1/Ppo5 siRNA Matches	PhL/R1 siRNA Matches	Invertase siRNA Matches
Human (9606)	163,241	0	1	0
Potato (4113)	33,238	324	466	318

The number of 21-mers with complete complementarity to an annotated transcript are shown for each inverted repeat (Asn1/Ppo5, PhL/R1, and VInv) by organism (taxonomy identifier).

Table 33. Summary of Matches in Human Transcriptome

pSIM1278 siRNA (5' to 3')	Accession Number	Annotated Function
CCCUUCACACUUUAUUUAUUU	XR_913817	Uncharacterised noncoding RNA

Genomic DNA Database Searches

In addition to an assessment of RNA databases, the potential siRNA derived from the two pSIM1278 inverted repeats and the VInv inverted repeat in pSIM1678 were queried against the NCBI database containing the human genomic sequence (Table 34 and Table 35).

A few matches were associated with intergenic regions, which are unannotated regions located between genes within the genome. Even fewer hits were associated with protein coding genes, but the region of complementarity was associated specifically with introns. There were no siRNA target sites associated with exons in protein coding genes. A match corresponding to a single non-coding RNA was identified in the human genome. This match was also previously identified in the RNA database analysis (Table 33) and described (Table 34). These findings are consistent with the results of the RNA database analysis as introns are not present in the mature mRNA contained in the transcript database and targeted by small RNA for down regulation.

Table 34. Summary of Matches Between pSIM1278 siRNA and the Human Genome

Organism	Protein Coding Genes		Other Loci	
	Introns	Exons	Non-coding RNA	Intergenic Regions*
Human	10	0	1	14

*Intergenic regions refer to positive matches against the genome that do not correspond to any known genes.

Table 35. Summary of Matches Between pSIM1678 siRNA and the Human Genome

Organism	Protein Coding Genes		Other Loci	
	Introns	Exons	Non-coding RNA	Intergenic Regions*
Human	1	0	0	2

Collectively, the query of the potential siRNA against the genomic database confirmed earlier analyses based upon annotated RNA transcripts. The only match in the human genome was to non-coding RNA. The bioinformatic assessment provides additional evidence that the consumption of small RNA present in W8, X17, and Y9 potatoes is as safe as the consumption of conventional potatoes.

Conservation of the Vacuolar Invertase Gene Sequence is Limited to Plants

As previously mentioned, in addition to the down regulated potato transcripts in the events authorised by the Health Canada (E12, F10, J3, and J55), W8, X17, and Y9 contain sequence that down regulates the potato vacuolar invertase through RNAi. Bioinformatic analysis demonstrated the lack of complementarity between the potential siRNA in W8, X17, and Y9 and the genome and transcriptome of humans.

To further demonstrate the safety of the additional VInv down-regulation cassette in W8, X17, and Y9, a BLAST search of the NCBI nucleotide database of the potato *VInv* complete coding mRNA sequence (DQ478950) was performed. This analysis was conducted to confirm that there are no significant sequence similarities between the human genome and the potato *VInv* sequence used for down regulation.

These results show that conservation of the potato *VInv* gene sequence is limited to plants (Figure 54); thus, potatoes containing small RNA directed against this gene are as safe for consumption as conventional potatoes.

Figure 54.Similarity Between Plant Invertase Sequences

BLAST search of the NCBI nucleotide database using the potato invertase complete coding mRNA sequence (DQ478950) returned 128 hits*. Invertase (*VInv*) sequences are coloured and labelled according to species. Evolutionary relatedness was inferred on 91 sequences by the Neighbour-Joining method using MEGA6 (Nei and Naruya, 1987; Tamura et al., 2013). The tree is drawn to scale, with branch lengths in units of nucleotide base differences per site (nbdps), the same units as the evolutionary distances used to infer the phylogenetic tree.

*Expect threshold was set to 10 and the maximum target hits was set to 20,000. All large genome or BAC sequences >10,000 bp were excluded, as well as all partial sequences <600 bp, resulting in 91 sequences.

Dietary Exposure of siRNA to Humans

A dietary risk assessment provided substantial evidence that siRNA from W8, X17, and Y9 potatoes would present both minimal hazard and negligible exposure. This included the history of safe use of small RNA in the diets of humans, numerous biological barriers to uptake, and lack of complementarity to human transcripts, as discussed above. Nevertheless, the levels of siRNA in W8, X17, and Y9 tubers and the amount of siRNA consumed by humans were estimated, measuring the total population of siRNA produced by the inserts from pSIM1278 and pSIM1678. The data for event W8 is presented.

To conduct the human exposure assessments, the estimated levels of siRNA in W8 potatoes were compared to the 95th percentile potato consumption rates in the United States. These results are consistent for X17 and Y9, which are transformed with the same two plasmids.

Human exposure calculations reflect the 95th percentile potato consumption from the Dietary Exposure Evaluation Model – Food Commodity Intake Database (DEEM-FCID)(EPA, 2014). These are consumption levels of people who eat more potatoes and potato-containing products than 95% of the population. Potato consumption in DEEM includes a wide variety of potato sources, such as whole tubers (which includes fries, chips, dry flakes, flour) and components of potatoes present in infant food. While the consumption data that form the basis of the DEEM-FCID model are derived from a United States survey, the data are applicable to other populations.

Based on DEEM exposure (95th percentile), the United States total population has a potato consumption rate of 2.85 g/kg body weight per day (bw/d) and adults aged 20-49 have a potato consumption rate of 2.37 g/kg bw/d. The sub-population with the highest consumption of potatoes is children aged 1 to 2 years with a consumption of 7.14 g/kg bw/d (Table 25).

The human exposure assessment used the DEEM-FCID potato consumption data (Table 25) and our estimates for the amount of siRNA in W8, X17, and Y9 tubers. The amount of siRNA in tubers was calculated using the following data. These data would apply equally to X17 and Y9 tubers.

- The amount of total RNA in tubers is 280 µg/g dry weight, based on Jonas et al., 2001.
- Estimations for siRNA exposure were derived from small RNA constitutively expressed in tobacco (Chau and Lee, 2007)
    - In their system, 7.5% of the total small RNA is the population of transgene-derived siRNA. Therefore, the amount of total transgene-derived siRNA in W8 potatoes is estimated to be 0.042 µg/g dry weight, or 0.015% of the total RNA.

These values, along with high-end potato consumption rates, were used to calculate a conservative estimate of dietary exposure to the total amount of transgene-derived siRNA in W8 potatoes.

The exposure estimate for total siRNA from W8 tubers was 120 ng/kg bw/d for the total United States population, 100 ng/kg bw/d for adults aged 20-49, and 300 ng/kg bw/d for children aged 1-2 years (Table 36).

Table 36. Human Margins of Exposure for Total W8 siRNA

DEEM Population	Potato Consumption at 95th Percentile	Estimated Exposure to Total siRNA	Margin of Exposure Compared to Petrick et al. (2015): 48 mg/kg bw/d
Total United States population	2.85 g/kg bw/d	120 ng/kg bw/d	401,003
Children Ages 1-2	7.14 g/kg bw/d	300 ng/kg bw/d	160,064
Adults Ages 20-49	2.37 g/kg bw/d	100 ng/kg bw/d	482,218

To place the concentration of siRNA in W8 potatoes in context, a margin of exposure (MOE) was calculated. This used a No Observable Adverse Effects Level (NOAEL) of 48 mg/kg/day based on the maximum dose of siRNA duplex administered to mice in a 28-day feeding study that used the OECD 407 guidelines (Petrick et al., 2015). This study is the most relevant because mice were gavaged with four unique siRNA duplexes with 100% complementarity to the mouse *vATPase* gene. The authors did not

identify any biological activity or adverse effects in the 28-day repeated dose gavage assay at the highest dose tested (48 mg/kg bw/d). These siRNAs were known to have biological activity when introduced directly into mouse kidney cells, because 70-80% depletion of mouse *vATPase* mRNA was observed. Despite this high level of down regulation, significant cytotoxicity was not observed in the kidney cell assay.

This dietary exposure assessment is based on a number of conservative assumptions. The estimate uses values from 95th percentile consumers of potato and assumes all of the potatoes consumed in the diet are W8 potatoes. It also assumes that all of siRNA was resistant to degradation during cooking and processing. In this assessment, a MOE of 160,064 was calculated for potatoes consumed in the subgroup with the highest estimated potential exposure (children aged 1-2). This means that children aged 1-2 would have to consume approximately 12,571 kg of potatoes each day (MOE × kg/consumption/day) to reach an equivalent NOAEL dose of siRNA identified in the Petrick et al. (2015) study. Generally, margins greater than 1000 are adequate as a basis for recommending no further action (Health Canada, 2004).

These estimates point to the negligible human exposure to siRNA. The lack of a pathway to harm from siRNA consumed in food further supports the close to zero risk attributed by regulatory agencies (Section 9.1; U.S. EPA, U.S. FDA, FSANZ) to consumption of siRNA.

Sufficient evidence of negligible exposure existed to reach a risk assessment determination without further data or studies.

Conclusion of RNAi Safety

According to scientific literature, RNA and siRNA are labile during processing and digestion and biological barriers further reduce potential exposure by limiting uptake of siRNA into the cells of mammals (Fabre et al., 2014; Hickerson et al., 2008; McAllan, 1982). There is no mechanism for harm in consuming siRNA in W8, X17, and Y9 potatoes due to RNA lability during processing and digestion, extensive biological barriers that limit uptake and activity in cells, and the lack of complementarity between the potential siRNA in W8, X17, and Y9 and the transcriptome and genome of humans. In addition, conservation of the potato *VInv* gene is limited to plants, providing additional evidence that potatoes containing small RNA directed against the potato *VInv* gene are as safe for consumption as conventional potatoes.

Based on the estimated exposure analysis, humans have very high margins of exposure for the siRNA consumed from W8, X17, and Y9 tubers. Given that small RNA are ubiquitous in nature, present in all food, and unlikely to accumulate in the environment, consumption of W8, X17, and Y9 potatoes and their associated siRNA is as safe as the consumption of conventional potatoes.

B.3(d)(i) where RNA interference has been used: the role of any endogenous target gene and any changes to the food as a result of silencing that gene

Glucose and fructose can accumulate in tubers during cold storage in a process known as cold-induced sweetening (Bhaskar et al., 2010). If potatoes contain high levels of reducing sugars, they can become undesirably dark and develop bitter flavours after frying (Halterman et al., 2016).

Four of the potato transcripts targeted for reduced expression are: asparagine synthetase, phosphorylase-L, water dikinase, and polyphenol oxidase. The aim of the suppression of asparagine synthetase is to reduce levels of free asparagine and the aim of suppression of PhL and R1 is to reduce levels of the reducing sugars, fructose and glucose. Collectively, the reduction of free asparagine and reducing sugars results in potato tubers with reduced acrylamide potential. Reduced expression of polyphenol oxidase results in tubers with reduced blackspot bruising. The down regulation of these genes has been assessed by FSANZ (Application 1128).

The potato vacuolar invertase (VINV) is also targeted for reduced expression through RNAi, resulting in lower reducing sugars. Following starch breakdown in the amyloplast, glucose, glucose-6-phophsate, and maltose are transferred to the cytoplasm. From there, the sugars are further metabolised and shuttled into the glycolysis pathway for mitochondrial respiration or converted into sucrose (Malone et al., 2006; Sowokinos, 2001). Invertase enzymes including vacuolar invertase (VINV) hydrolyse the sucrose into glucose and fructose. Down regulation of VINV reduces the conversion of sucrose to fructose and glucose during cold storage, which results in lower levels of acrylamide upon frying and inhibits formation of sugar-related defects in fries and chips (Halterman et al., 2016; Ye et al., 2010).

Lower free asparagine and lower reducing sugars are within the range for conventional potatoes and are considered substantially equivalent to edible potatoes (Section B5).

B.3(d)(ii) where RNA interference has been used: the expression levels of the RNA transcript

The expression levels of transcripts from the 5 potato enzymes are presented in Section A.3(g).

The reduced expression of asparagine synthetase, polyphenol oxidase, and vacuolar invertase were consistent with the compositional and trait efficacy data in Sections B.1 and B.5. Although the down regulation of phosphorylase L and water dikinase transcripts was less effective, the intended trait of lower reducing sugars is still prevalent in these events from the down regulation of vacuolar invertase, as shown in the trait efficacy assessment (Section B.1(a)).

B.3(d)(iii) where RNA interference has been used: the specificity of the RNA interference

The reduced expression of the 5 potato genes is facilitated by the *Agp* promotor of the ADP glucose pyrophosphorylase gene (*Agp*) and the *Gbss* promoter of the granule-bound starch synthase gene (*Gbss*). Both promotors are primarily active in tubers. The specificity of reduced expression is demonstrated in Section A.3(g).

B.5 Compositional analyses of the food produced using gene technology

This must include all of the following:

B.5(a) the levels of relevant key nutrients, toxicants and anti-nutrients in the food produced using gene technology compared with the levels in an appropriate comparator (usually the non-GM counterpart). A statistical analysis of the data must be provided.

B.5(b) information on the range of natural variation for each constituent measured to allow for assessment of biological significance should any statistically significant differences be identified

B.5(c) the levels of any other constituents that may potentially be influenced by the genetic modification, as a result, for example, of downstream metabolic effects, compared with the levels in an appropriate comparator as well as the range of natural variation.

Compositional Assessment of W8, X17, and Y9

Compositional analysis of W8, X17, and Y9 was conducted to evaluate the levels of key nutrients (proximates, vitamins, amino acids, and minerals) and glycoalkaloids compared to their non-transformed control varieties. In addition, composition analysis is provided for the primary events F10 (Report15-61-SPS-COMP) and J3 (Report15-62-SPS-COMP).

The compositional assessments evaluated:

1. Proximates, vitamins, and minerals (Table 37, Table 38 and Table 39);
2. Total amino acids (Table 40, Table 41 and Table 42); and
3. Glycoalkaloids (Table 43, Table 44 and Table 45).

Analytes were measured from tubers harvested from field trials conducted for phenotypic and agronomic assessments. Additional details for the composition and statistical methods can be found in:

- Report15-47-SPS-COMP-RPT, W8;
- Report15-51-SPS-COMP-RPT, X17; and
- Report15-112-SPS-COMP-RPT, Y9.

The analytes selected for the compositional assessment were based on the recommendations in the OECD consensus document on compositional considerations for new varieties of potatoes: key nutrients, anti-nutrients, and toxicants (OECD, 2002).

Additional potato varieties were grown as reference material to provide a range of values common to conventional potatoes. The reference varieties are commonly used in the chip, fry, dehydrated, or fresh markets. Reference materials were used to calculate tolerance intervals. Tolerance intervals were used for compositional data to represent the natural variability among potatoes. The tolerance interval attempts to predict the range in which most values of a population will fall (Vardeman, 1992). The tolerance intervals were calculated to contain, with 95% confidence, 99% of the values in a population. The control varieties were used in the tolerance interval calculation because of their widespread popularity and history of safe use. The inclusion of the controls in the tolerance interval did not affect the statistical analysis because the tolerance interval was a separate calculation.

The compositional results from W8, X17, and Y9 were compared to this tolerance interval and the combined range of values for each analyte available from the published literature. In interpreting the data, emphasis was placed on the analyte means. Means that fell within the tolerance interval and/or CLR for the analyte were considered to be within the normal variability of commercial potato varieties.

Detailed compositional analysis results for W8, X17, and Y9 is described and summarised below.

Proximates, Vitamins, and Minerals

Mean values of proximate, vitamin, and mineral levels in W8 were within the tolerance interval and/or combined literature range. Statistical differences between W8 and Russet Burbank were seen for vitamin C and vitamin B6 (Table 37). However, the observed levels are within the normal range of compositional variation and therefore are not nutritionally meaningful.

Table 37. Proximates, Vitamins, and Minerals in W8 and Russet Burbank

Variable	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval	CLR[2]
						Min	Max		
Protein (%)	W8	2.11	0.6444	0.179	32	1.83	2.58	1.53-2.79	0.700-4.60
	Russet Burbank	2.13		0.191	32	1.82	2.50		
Fat (%)	W8	0.162	0.8616	0.0508	32	0.100	0.250	0.100-0.45	0.0200-.200
	Russet Burbank	0.166		0.0841	32	0.100	0.460		
Ash (%)	W8	0.958	0.8664	0.150	32	0.643	1.26	0.461-1.41	0.440-1.90
	Russet Burbank	0.951		0.153	32	0.717	1.49		
Crude Fibre (%)	W8	0.469	0.2647	0.0891	32	0.305	0.670	0.190-0.740	0.170-3.50
	Russet Burbank	0.438		0.0926	32	0.305	0.660		
Carbohydrates (%)	W8	16.5	0.0577	1.59	32	13.3	20.0	13.2-22.1	13.3-30.53
	Russet Burbank	17.2		1.41	32	14.5	19.4		
Calories (kcal/100 g)	W8	75.7	0.0599	6.26	32	63.7	89.4	63.5-97.9	80.0-110
	Russet Burbank	78.8		5.12	32	68.8	87.7		
Moisture (%)	W8	80.3	0.0652	1.44	41	76.9	83.3	75.0-83.4	63.2-86.9
	Russet Burbank	79.7		1.17	41	77.6	82.0		
Vitamin B3 (mg/100 g)	W8	1.86	0.8651	0.225	32	1.41	2.30	0.768-2.86	0.0900-3.10
	Russet Burbank	1.84		0.258	32	1.43	2.48		
Vitamin B6 (mg/100 g)	W8	0.120	**0.0019**	0.0133	32	0.096	0.150	0.074-0.150	0.110-0.340
	Russet Burbank	0.132		0.0104	32	0.111	0.150		
Vitamin C (mg/100 g)	W8	26.7	**0.0040**	3.59	32	18.7	32.2	11.9-44.5	1.00-54.0
	Russet Burbank	23.5		3.23	32	16.9	28.8		
Copper (mg/100 g)	W8	0.0695	0.4460	0.0172	32	0.05	0.12	0.050-0.130	0.020-0.700
	Russet Burbank	0.0724		0.0180	32	0.05	0.11		
Magnesium mg/100 g)	W8	20.9	0.1472	2.42	32	17.3	29.1	13.3-29.0	11.3-55.0
	Russet Burbank	20.1		1.83	32	16.4	23.5		
Potassium (mg/100 g)	W8	427	0.9262	28.5	32	367	481	281-578	350-625
	Russet Burbank	428		30.2	32	367	484		

[1]P-values indicating significant differences with control are underlined and in bold.
[2]Combined Literature Ranges are from Horton and Anderson, 1992; Lisinska and Leszczynski, 1989; Rogan et al., 2000; Talburt et al., 1987.

Mean values of proximate, vitamin, and mineral levels in X17 were within the tolerance interval and/or combined literature range. Statistical differences between X17 and Ranger Russet were seen for crude fibre, vitamin C, and potassium (Table 38). However, the levels are within the normal range of compositional variation and therefore are not nutritionally meaningful.

Table 38. Proximates, Vitamins, and Minerals in X17 and Ranger Russet

Variable	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval	CLR[2]
						Min	Max		
Protein (%)	X17	2.32	0.6602	0.150	32	2.06	2.57	1.49-3.02	0.700-4.60
	Ranger Russet	2.30		0.188	32	1.93	2.66		
Fat (%)	X17	0.151	0.9889	0.0420	32	0.100	0.250	0.100-0.397	0.0200-.200
	Ranger Russet	0.150		0.0510	32	0.100	0.320		
Ash (%)	X17	0.961	0.1406	0.166	32	0.735	1.37	0.450-1.42	0.440-1.90
	Ranger Russet	0.899		0.223	32	0.287	1.27		
Crude Fibre (%)	X17	0.591	**0.0242**	0.0950	32	0.449	0.820	0.212-.807	0.170-3.50
	Ranger Russet	0.533		0.0800	32	0.406	0.690		
Carbohydrates (%)	X17	20.2	0.1210	2.10	32	15.4	24.9	12.4-24	13.3-30.53
	Ranger Russet	19.6		1.94	32	15.9	25.1		
Calories (kcal/100 g)	X17	91.3	0.1402	8.07	32	71.0	109	59.8-106	80.0-110
	Ranger Russet	89.0		7.44	32	73.8	110		
Moisture (%)	X17	76.6	0.1346	2.02	32	72.0	81.4	72.6-84.3	63.2-86.9
	Ranger Russet	77.1		1.87	32	71.8	81.0		
Vitamin B3 (mg/100 g)	X17	2.38	0.7212	0.219	32	1.98	2.82	0.721-3.05	0.0900-3.10
	Ranger Russet	2.41		0.184	32	2.07	2.83		
Vitamin B6 (mg/100 g)	X17	0.120	0.3832	0.0100	32	0.108	0.150	0.077-0.157	0.110-0.340
	Ranger Russet	0.117		0.0120	32	0.104	0.150		
Vitamin C (mg/100 g)	X17	41.0	**0.0007**	4.73	32	32.0	48.1	11.2-45.7	1.00-54.0
	Ranger Russet	35.8		7.18	32	20.0	47.0		
Copper (mg/100 g)	X17	0.0931	0.1868	0.0540	32	0.050	0.290	0.0500-0.251	0.020-0.700
	Ranger Russet	0.106		0.107	32	0.050	0.640		
Magnesium (mg/100 g)	X17	23.6	0.9376	1.89	32	20.2	28.1	14.2-28.8	11.3-55.0
	Ranger Russet	23.6		1.62	32	20.1	27.0		
Potassium (mg/100 g)	X17	463	**0.0475**	55.0	32	378	583	281-590	350-625
	Ranger Russet	444		57.5	32	362	550		

[1]P-values indicating significant differences with control are underlined and in bold.
[2]Combined literature ranges are from OECD, 2002; Rogan et al., 2000; Talley et al., 1984.

Mean values of proximate, vitamin, and mineral levels in Y9 were within the tolerance interval and/or combined literature range. Statistical differences between Y9 and Atlantic were seen for protein, crude fibre, carbohydrates, calories, moisture, and potassium (Table 39). However, the levels are within the normal range of compositional variation and therefore not nutritionally meaningful.

Table 39. Proximates, Vitamins, and Minerals in Y9 and Atlantic

Variable	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval	CLR[2]
						Min	Max		
Protein (%)	Y9	2.52	**0.0035**	0.217	28	2.14	2.88	1.49-3.02	0.700-4.60
	Atlantic	2.42		0.281	28	1.90	2.93		
Fat (%)	Y9	0.131	0.5054	0.0480	28	0.100	0.250	0.100-0.397	0.0200-.200
	Atlantic	0.145		0.0690	28	0.100	0.340		
Ash (%)	Y9	0.905	0.2192	0.192	28	0.464	1.22	0.450-1.42	0.440-1.90
	Atlantic	0.968		0.128	28	0.707	1.22		
Crude Fibre (%)	Y9	0.469	**0.0432**	0.108	28	0.326	0.690	0.212-.807	0.170-3.50
	Atlantic	0.408		0.0930	28	0.233	0.620		
Carbohydrates (%)	Y9	20.6	**0.0107**	2.53	28	16.7	24.7	12.4-24	13.3-30.53
	Atlantic	19.7		2.54	28	15.2	23.8		
Calories (kcal/100 g)	Y9	93.3	**0.0067**	10.2	28	77.6	111	59.8-106	80.0-110
	Atlantic	89.4		10.1	28	73.0	107		
Moisture (%)	Y9	75.9	**0.0090**	2.59	28	71.4	79.8	72.6-84.3	63.2-86.9
	Atlantic	76.8		2.51	28	72.4	81.0		
Vitamin B3 (mg/100 g)	Y9	1.69	0.408	0.165	28	1.45	2.03	0.721-3.05	0.0900-3.10
	Atlantic	1.64		0.216	28	1.34	2.22		
Vitamin B6 (mg/100 g)	Y9	0.118	0.8428	0.0110	28	0.103	0.140	0.077-0.157	0.110-0.340
	Atlantic	0.117		0.0120	28	0.098	0.140		
Vitamin C (mg/100 g)	Y9	25.5	0.926	4.90	28	16.1	35.1	11.2-45.7	1.00-54.0
	Atlantic	25.5		5.99	28	11.9	36.6		
Copper (mg/100 g)	Y9	0.100	0.9053	0.039	28	0.057	0.250	0.050-0.251	0.020-0.700
	Atlantic	0.099		0.032	27	0.057	0.210		
Magnesium (mg/100 g)	Y9	20.3	0.6805	0.993	28	18.3	22.6	14.2-28.8	11.3-55.0
	Atlantic	20.2		1.38	28	17.2	22.5		
Potassium (mg/100 g)	Y9	466	**0.0461**	40.0	28	401	541	281-590	350-625
	Atlantic	453		41.7	28	355	511		

[1]P-values indicating significant differences with control are underlined and in bold.
[2]Combined Literature Ranges are from Horton and Anderson, 1992; Lisinska and Leszczynski, 1989; Rogan et al., 2000; Talburt et al., 1987.

Mean values of proximate, vitamin, and mineral levels were measured in W8, X17, and Y9 tubers. Although statistically significant differences were observed in each event, the mean values of proximates, vitamins, and minerals were within the tolerance interval and/or combined literature range. Therefore, W8, X17, and Y9 are nutritionally equivalent to conventional potatoes in proximates, vitamins, and mineral levels.

Total Amino Acids

Amino acid levels were measured in W8, X17, and Y9 tubers (Table 40, Table 41, and Table 42). Statistically significant differences were observed in amino acid levels in each event. However, all mean values for total amino acids were within the tolerance interval and/or combined literature range. Therefore, the levels are within the normal range of compositional variation and not nutritionally meaningful. The events W8, X17, and Y9 are equivalent to conventional potatoes in total amino acids.

Table 40. Total Amino Acids in W8 and Russet Burbank

Variable	Variety	Mean (mg/100g)	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval	CLR[2]
						Min	Max		
Alanine	W8	70.4	**0.0004**	7.32	32	58.0	87.7	38.9-95.1	39.2-95.2
	Russet Burbank	63.8		7.04	32	41.3	75.2		
Arginine	W8	104	0.1463	16.6	32	83.7	157	52.9-142	70.0-138
	Russet Burbank	98.4		13.2	32	62.5	119		
Aspartic Acid and Asparagine	W8	255	**<.0001**	31.3	32	189	331	203-686	339-738
	Russet Burbank	454		77.5	32	278	636		
Cystine	W8	23.4	**<.0001**	3.45	32	15.6	30.5	10.2-32.2	48.0-92.5
	Russet Burbank	18.9		3.43	32	11.5	26.3		
Glutamic Acid and Glutamine	W8	478	**<.0001**	55.2	32	389	583	185-482	292-604
	Russet Burbank	310		32.7	32	232	373		
Glycine	W8	58.1	**0.0079**	6.83	32	45.7	70.6	38.5-76.3	46.0-97.5
	Russet Burbank	52.8		5.35	32	40.8	62.4		
Histidine	W8	33.1	0.7008	3.57	32	26.2	41.6	18.4-46.8	13.3-46.9
	Russet Burbank	32.7		4.61	32	24.2	42.7		
Isoleucine	W8	72.5	0.5519	7.32	32	60.0	95.3	46.9-98.4	52.5-95.3
	Russet Burbank	71.2		8.55	32	47.9	86.9		
Leucine	W8	110	**0.0162**	11.8	32	81.4	137	67.2-149	68.5-138
	Russet Burbank	102		11.1	32	72.1	123		
Lysine	W8	100	0.1590	8.47	32	83.6	118	63.7-136	68.7-137
	Russet Burbank	95.7		8.18	32	73.2	115		
Methionine	W8	41.4	0.2150	4.87	32	34.2	51.6	24.7-52.0	28.5-50.0
	Russet Burbank	39.9		4.47	32	29.8	49.4		
Phenylalanine	W8	81.9	0.7842	7.35	32	68.4	100	55.5-115	55.2-109
	Russet Burbank	81.2		8.53	32	54.5	94.8		
Proline	W8	63.1	**0.0399**	13.4	32	23.2	104	11.0-127	35.5-146
	Russet Burbank	52.9		15.1	32	11.8	72.9		
Serine	W8	74	**0.0175**	8.05	32	56.8	91.5	48.5-94.0	50.0-102
	Russet Burbank	68.7		6.61	32	49.1	80.1		
Threonine	W8	72.9	**0.0020**	8.59	32	56.0	91.5	41.8-95.2	43.6-85.5
	Russet Burbank	65.3		7.12	32	46.7	79.2		
Tryptophan	W8	21.5	0.7800	2.15	32	17.4	26.8	13.5-29.1	11.4-28.2
	Russet Burbank	21.9		2.57	32	16.1	26.8		
Tyrosine	W8	80.3	**<.0001**	6.76	32	68.8	95.1	39.3-96.5	45.7-94.2
	Russet Burbank	65.2		7.74	32	47.3	77.7		
Valine	W8	112	0.2126	11.5	32	93.0	141	67.7-143	75.2-145
	Russet Burbank	108		12.6	32	73.6	127		

[1]P-values indicating significant differences with controls are underlined and in bold.
[2]Combined literature ranges are from OECD, 2002; Rogan et al., 2000; Talley et al., 1984.

Table 41. Total Amino Acids in X17 and Ranger Russet

Variable	Variety	Mean (mg/100g)	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval	CLR[2]
						Min	Max		
Alanine	X17	83.3	**<.0001**	6.67	32	76.5	100	41.8-96.1	39.2-95.2
	Ranger Russet	69.1		5.90	32	60.2	81.8		
Arginine	X17	155	**<.0001**	26.8	32	123	223	36.5-182	70.0-138
	Ranger Russet	125		19.2	32	98.5	174		
Aspartic Acid and Asparagine	X17	281	**<.0001**	27.6	32	225	332	229-708	339-738
	Ranger Russet	509		47.9	32	437	602		
Cystine	X17	26.6	**0.0024**	3.29	32	21.4	33.6	7.89-39.5	48.0-92.5
	Ranger Russet	23.3		3.43	32	17.6	30.7		
Glutamic Acid and Glutamine	X17	458	**<.0001**	34.5	32	400	546	184-507	292-604
	Ranger Russet	319		25.9	32	255	370		
Glycine	X17	70.8	**<.0001**	7.89	32	56.1	89.0	32.7-91.4	46.0-97.5
	Ranger Russet	61.5		7.37	32	47.7	76.8		
Histidine	X17	35.4	0.0642	3.28	32	30.3	43.7	19.3-47.3	13.3-46.9
	Ranger Russet	33.5		3.32	32	28.5	40.3		
Isoleucine	X17	83.8	**0.0060**	7.82	32	70.6	99.3	46.5-106	52.5-95.3
	Ranger Russet	78.1		8.04	32	58.3	90.6		
Leucine	X17	137	**<.0001**	14.4	32	108	170	57.4-176	68.5-138
	Ranger Russet	118		12.9	32	92.1	144		
Lysine	X17	118	**0.0003**	8.46	32	103	135	61.4-149	68.7-137
	Ranger Russet	107		7.41	32	88.1	120		
Methionine	X17	42.5	**0.0437**	3.17	32	37.1	49.8	25.6-52.6	28.5-50.0
	Ranger Russet	40.2		3.59	32	34.1	47.5		
Phenylalanine	X17	97.9	**0.0039**	8.52	32	82.9	116	53.2-125	55.2-109
	Ranger Russet	91.2		9.22	32	75.9	109		
Proline	X17	87.4	**0.0290**	14.8	32	70.4	132	17.9-132	35.5-146
	Ranger Russet	77.4		17.0	32	58.1	125		
Serine	X17	90.1	**<.0001**	7.06	32	78.6	109	46.3-104	50.0-102
	Ranger Russet	77.9		7.29	32	65.5	93.0		
Threonine	X17	87.8	**<.0001**	7.55	32	77.2	107	38.3-109	43.6-85.5
	Ranger Russet	74.9		6.70	32	62.7	89.4		
Tryptophan	X17	24.7	0.3053	3.55	32	19.6	32.3	13.3-31.8	11.4-28.2
	Ranger Russet	23.4		3.59	32	17.0	30.8		
Tyrosine	X17	87.2	**<.0001**	7.57	32	72.1	104	37.8-106	45.7-94.2
	Ranger Russet	74.0		5.75	32	63.4	86.1		
Valine	X17	114	**0.0021**	7.75	32	102	129	71.5-141	75.2-145
	Ranger Russet	106		9.21	32	86.0	122		

[1]P-values indicating significant differences with controls are underlined and in bold.
[2]Combined literature ranges are from OECD, 2002; Rogan et al., 2000; Talley et al., 1984.

Table 42. Total Amino Acids in Y9 and Atlantic

Variable	Variety	Mean (mg/100g)	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval	CLR[2]
						Min	Max		
Alanine	Y9	86.8	**<.0001**	9.51	28	72.9	109	41.8-96.1	39.2-95.2
	Atlantic	74.6		9.33	28	59.4	96.9		
Arginine	Y9	142	**<.0001**	19.3	28	112	196	36.5-182	70.0-138
	Atlantic	127		19.5	28	102	174		
Aspartic Acid and Asparagine	Y9	304	**<.0001**	31.4	28	256	360	229-708	339-738
	Atlantic	499		69.3	28	350	619		
Cystine	Y9	35.3	**0.0086**	3.02	28	30.7	42.1	10.0-39.5	48.0-92.5
	Atlantic	32.3		3.27	28	28.1	39.1		
Glutamic Acid and Glutamine	Y9	527	**<.0001**	55.5	28	417	603	184-507	292-604
	Atlantic	375		48.4	28	266	467		
Glycine	Y9	87.0	**<.0001**	9.51	28	73.3	110	32.7-91.4	46.0-97.5
	Atlantic	76.4		9.30	28	61.8	96.7		
Histidine	Y9	36.8	**0.0458**	3.10	28	30.9	43.7	19.3-47.3	13.3-46.9
	Atlantic	34.6		4.65	28	27.3	44.5		
Isoleucine	Y9	92.6	**<.0001**	9.14	28	80.9	113	46.5-106	52.5-95.3
	Atlantic	83.9		9.91	28	70.0	108		
Leucine	Y9	169	**<.0001**	19.1	28	142	218	57.4-176	68.5-138
	Atlantic	147		18.8	28	120	190		
Lysine	Y9	130	0.0745	19.1	28	85.6	173	61.4-149	68.7-137
	Atlantic	120		21.2	28	47.3	157		
Methionine	Y9	41.5	0.1298	5.06	28	28.1	52.6	25.6-52.6	28.5-50.0
	Atlantic	40.0		4.39	28	32.7	48.7		
Phenylalanine	Y9	111	**<.0001**	11.7	28	92.4	140	53.2-125	55.2-109
	Atlantic	100.0		12.1	28	82.9	125		
Proline	Y9	109	**0.0006**	19.7	28	77.5	153	17.9-132	35.5-146
	Atlantic	89.1		11.1	28	59.9	113		
Serine	Y9	98.0	**<.0001**	10.3	28	81.9	123	46.3-104	50.0-102
	Atlantic	86.8		9.73	28	72.0	108		
Threonine	Y9	102	**<.0001**	11.5	28	84.9	132	38.3-109	43.6-85.5
	Atlantic	89.0		11.2	28	73.5	115		
Tryptophan	Y9	24.5	**0.0217**	2.42	28	20.7	29.5	13.3-31.8	11.4-28.2
	Atlantic	23.4		2.88	28	18.7	28.2		
Tyrosine	Y9	95.4	**<.0001**	10.0	28	80.8	121	37.8-106	45.7-94.2
	Atlantic	82.5		10.3	28	67.8	107		
Valine	Y9	121	**<.0001**	11.6	28	104	146	71.5-141	75.2-145
	Atlantic	108		12.2	28	90.2	136		

[1]P-values indicating significant differences with controls are underlined and in bold.
[2]Combined literature ranges are from OECD, 2002; Rogan et al., 2000; Talley et al., 1984.

Glycoalkaloids in W8, X17, and Y9

Glycoalkaloids are toxins commonly found in Solanaceous crops, including potato with 95% of the total glycoalkaloids consisting of α-solanine and α-chaconine (OECD, 2002). The widely accepted safety limit for total glycoalkaloids in tubers is 20 mg/100 gm fresh weight (Smith et al. 1996; Health Canada, 2012b). The mean concentrations of glycoalkaloids in W8, X17, and Y9 were not statistically different from their corresponding non-transformed varietal controls and were well below the Canadian standard (Table 43, Table 44, and Table 45).

Table 43. Tuber Glycoalkaloid Levels in W8

Variety	Mean (mg/100g)	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval	CLR[2]
					Min	Max		
W8	7.20	0.1615	3.64	32	5	19.0	5.00-14.4	3.20-210.4
Russet Burbank	6.40		2.18	32	5	13.2		

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

Table 44. Tuber Glycoalkaloid Levels in X17

Variety	Mean (mg/100g)	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval	CLR[2]
					Min	Max		
X17	7.38	0.7555	1.79	32	4.66	11.0	5.00-14.6	3.20-210.4
Ranger Russet	7.21		2.08	32	4.07	13.8		

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

Table 45. Tuber Glycoalkaloid Levels in Y9

Variety	Mean (mg/100g)	P-Value[1]	Standard Deviation	N	Range		Tolerance Interval	CLR[2]
					Min	Max		
Y9	6.90	0.6276	2.90	28	3.93	14.4	5.00-14.6	3.20-210.4
Atlantic	7.27		4.33	28	4.13	22.8		

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

Patatin in W8, X17, and Y9

Potatoes are not among the "Big Eight" group of foods that account for ~90% of all food allergies in the U.S. (FARRP, 2014). There are a few reports of allergies to cooked potato in children (De Swert et al., 2002, 2007). However, most children with potato allergy develop tolerance at mean age of four years (De Swert, et al., 2007). Patatin (Sol t 1) has been identified as the primary allergen involved in this reaction (Astwood et al., 2000). Patatin is a storage glycoprotein that displays lipase activity and makes up about 40% of the soluble protein in tubers (Mignery et al., 1988). Because potato protein naturally contains a relatively large proportion of patatin, any unexpected change in patatin levels would be unlikely to affect allergenicity enough to alter consumption patterns for people allergic to potatoes. Additionally, there is no mechanistic reason to suggest that the level of patatin would be changed in W8, X17, and Y9.

Conclusion of the Compositional Assessment of W8, X17, and Y9

A thorough compositional assessment of key nutrients, including proximates, vitamins, minerals, and amino acids, and of glycoalkaloids was conducted for W8, X17, and Y9. Statistically significant differences were observed in the levels of some proximates, vitamins, minerals, and amino acids in W8, X17, and Y9. However, the observed levels were within the tolerance interval and/or combined literature range, demonstrating that the nutritional content of W8, X17, and Y9 falls within the normal range for potatoes. These results demonstrate that W8, X17, and Y9 are compositionally equivalent to Russet Burbank, Ranger Russet, and Atlantic, respectively, and to other commercial potato varieties.

Glycoalkaloid levels in W8, X17, and Y9 were unchanged and within the recommended safety limits. Because potato protein naturally contains a relatively large proportion of patatin, any unexpected change in patatin levels would not affect allergenicity enough to alter consumption patterns for people allergic to potatoes. The compositional assessment demonstrated that W8, X17, and Y9 are as safe and nutritious as conventional potatoes with a long history of safe consumption.

Reducing Sugars

Down regulation of water dikinase and phosphorylase slows the breakdown of starch into sugars in the amyloplast resulting in lower levels of fructose and glucose reducing sugars. The down regulation of invertase slows the breakdown of sucrose into glucose and fructose in the vacuole, resulting in decreased levels of glucose and fructose and increased sucrose levels in tubers. Sucrose, glucose and fructose levels were measured in events W8, X17 and Y9 and their parental variety at harvest and after storage at two temperatures (Tables 46–51).

Reducing Sugars in W8 Potatoes

Potatoes from W8 contain significantly lower levels of the reducing sugars, and higher levels of sucrose compared to Russet Burbank at harvest and after storage (

Table 46 and

Table 47). However, the mean concentrations of sucrose, glucose, and fructose were within the combined literature range and therefore within the normal range for potatoes. Lower levels of fructose and glucose and higher levels of sucrose in W8 compared to Russet Burbank is the result of reduced VInv activity.

Table 46. Russet Burbank and W8 Sugars in Tubers at Harvest and after Storage at 8 °C

Timing	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		CLR[2]
						Min	Max	
Fructose and Glucose (mg/100 g)								
Fresh	W8	38.4	**0.0002**	24.5	41	9.68	106	18.0-803
	Russet Burbank	146		98.3	41	14	406	
3 Months Storage	W8	122	**0.0056**	57.3	9	54.1	210	18.0-803
	Russet Burbank	483		114	9	298	598	
6 Months Storage	W8	116	**<.0001**	115	9	20.8	310	18.0-803
	Russet Burbank	261		106	9	153	459	
9 Months Storage	W8	106	**0.0320**	29.7	9	79.7	160	18.0-803
	Russet Burbank	224		91.7	9	105	372	
Sucrose (mg/ 100g)								
Fresh	W8	395	**<.0001**	141	41	161	775	39.7-1,390
	Russet Burbank	241		99.7	41	113	558	
3 Months Storage	W8	651	**<.0001**	82.2	9	520	738	39.7-1,390
	Russet Burbank	148		54.7	9	56.2	228	
6 Months Storage	W8	202	**0.0021**	18.8	9	177	229	39.7-1,390
	Russet Burbank	97.6		22.1	9	80.1	144	
9 Months Storage	W8	146	**<.0001**	29.1	9	105	201	39.7-1,390
	Russet Burbank	56.9		10.0	9	44.8	77.3	

[1]P-values indicating significant differences with controls are underlined and in bold.
[2]Literature range from Amrein et al., 2003; Vivanti et al., 2006.

Table 47. Russet Burbank and W8 Sugars in Tubers after Cold Storage at 3 °C

Timing	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		CLR[2]
						Min	Max	
Fructose and Glucose (mg/100 g)								
6 Months Storage	W8	91.7	**0.0002**	7.15	3	83.7	97.4	18-803
	Russet Burbank	640		74.8	3	590	726	
9 Months Storage	W8	151	**<.0001**	44	3	102	188	18-803
	Russet Burbank	754		45.2	3	703	788	
Sucrose (mg/ 100g)								
6 Months Storage	W8	963	**<.0001**	21.1	3	945	986	39.7-1,390
	Russet Burbank	182		37.9	3	138	206	
9 Months Storage	W8	645	**<.0001**	61.2	3	598	714	39.7-1,390
	Russet Burbank	152		13.6	3	137	163	

[1]P-values indicating significant differences with controls are underlined and in bold.
[2]Literature range from Amrein et al., 2003; Vivanti et al., 2006.

Reducing Sugars in X17 Potatoes

Potatoes from X17 contain significantly lower levels of the reducing sugars, fructose and glucose, and higher levels of sucrose compared to Ranger Russet at harvest and after storage (Table 48 and Table 49). However, the mean concentrations of sucrose, glucose, and fructose were within the combined literature range and therefore within the normal range for potatoes. Lower levels of fructose and glucose and higher levels of sucrose in X17 compared to Ranger Russet is the result of reduced VInv activity.

Table 48. Ranger Russet and X17 Sugars in Tubers at Harvest and after Storage at 8 °C

Timing	Variety	Mean	P-Value[1]	Standard Deviation	N	Range		CLR[2]
						Min	Max	
Fructose and Glucose (mg/100 g)								
Fresh	X17	21.2	**0.0196**	5.51	32	9.30	32.8	18.0-803
	Ranger Russet	85.8		43.3	32	31.8	183	
6 Months Storage	X17	74.2	**<.0001**	66.2	28	16.4	272	18.0-803
	Ranger Russet	220		129	28	89.7	594	
Sucrose (mg/100 g)								
Fresh	X17	326	**0.0317**	88.9	32	235	644	39.7-1,390
	Ranger Russet	256		63.0	32	209	433	
6 Months Storage	X17	298	**<.0001**	65.5	28	209	433	39.7-1,390
	Ranger Russet	172		47.1	28	102	354	

6 month storage data were not collected from the Adams County, Wisconsin site in 2013.
[1]P-values indicating significant differences with controls are underlined and in bold.
[2]Literature range from Amrein et al., 2003; Vivanti et al., 2006.

Table 49. Ranger Russet and X17 Sugars in Tubers after Cold Storage at 3 °C

Timing[1]	Variety	Mean[2]	P-Value[3]	Standard Deviation	N	Range		CLR[4]
						Min	Max	
Fructose and Glucose (mg/100 g)								
6 Months Storage	X17	289	**<.0001**	40.2	4	248	344	18.0-803
	Ranger Russet	889		120	4	755	1,047	
Sucrose (mg/100 g)								
6 Months Storage	X17	901	**<.0001**	117	4	752	1,030	39.7-1,390
	Ranger Russet	361		91.0	4	297	495	

[1]Data collected from the Minidoka County, Idaho site in 2013.
[2]The mean value of glucose and fructose measured in Ranger Russet after storage at 3°C was higher than what is recorded in the literature.
[3]P-values indicating significant differences with controls are underlined and in bold.
[4]Literature range from Amrein et al., 2003; Vivanti et al., 2006.

Reducing Sugars in Y9 Potatoes

Potatoes from Y9 contain significantly lower levels of the reducing sugars, fructose and glucose, and higher levels of sucrose compared to Atlantic at harvest and after storage (Table 50 and

Table 51). The mean reducing sugar levels in Y9 were below the literature range for conventional potatoes, which is expected given the intended trait. However, the mean concentrations of sucrose were within the combined literature range and therefore within the normal range for potatoes. Lower levels of fructose and glucose and higher levels of sucrose in Y9 compared to Atlantic is the result of reduced VInv activity.

Table 50. Atlantic and Y9 Sugars in Tubers at Harvest and after Storage at 10 °C

Timing	Variety	Mean[1]	P-Value[2]	Standard Deviation	N	Range		CLR[3]
						Min	Max	
Fructose and Glucose (mg/100 g)								
Fresh	Y9	7.43	**<.0001**	2.14	28	3.93	12.7	18.0-803
	Atlantic	20.1		9.49	28	7.46	39.4	
6 Months Storage	Y9	15.4	**0.0394**	15.7	28	6.40	58.7	18.0-803
	Atlantic	107		144	28	20.7	533	
Sucrose (mg/100 g)								
Fresh	Y9	169	**0.0386**	52.6	28	99.6	283	39.7-1,390
	Atlantic	150		38.1	28	96.3	225	
6 Months Storage	Y9	256	0.2533	207	28	127	839	39.7-1,390
	Atlantic	192		65.0	28	101	369	

[1]The mean value of glucose and fructose measured in Y9 at harvest and after storage at 10°C was lower than what is recorded in the literature.
[2]P-values indicating significant differences with controls are underlined and in bold.
[3]Literature Range from Amrein et al., 2003; Vivanti et al., 2006.

Table 51. Atlantic and Y9 Sugars in Tubers after Cold Storage at 3 °C

Variable[1]	Variety	Mean[2]	P-Value[3]	Standard Deviation	N	Range		CLR[4]
						Min	Max	
Fructose and Glucose (mg/100 g)								
6 Months Storage	Y9	11.8	**0.0017**	3.68	4	8.91	16.9	18.0-803
	Atlantic	35.7		7.47	4	25.6	42.4	
Sucrose (mg/100 g)								
6 Months Storage	Y9	132	0.1307	2.06	4	130	134	39.7-1,390
	Atlantic	162		40.5	4	134	222	

[1]Data collected from the Grant County, Washington site in 2014.
[2]The mean value of glucose and fructose measured in Y9 after storage at 3°C was lower than what is recorded in the literature.
[3]P-values indicating significant differences with controls are underlined and in bold.
[4]Literature ranges from Amrein et al., 2003; Vivanti et al., 2006.

Summary of Reducing Sugars in W8, X17 and Y9

As expected, levels of reducing sugars were lower at harvest and after storage, confirming the efficacy of the lower reducing sugars trait. In some cases, the mean reducing sugar levels were below the literature range for conventional potatoes, which is expected given the intended trait. The mean concentrations of sucrose were within the combined literature range and therefore within the normal range for potatoes.

Acrylamide Potential

Although acrylamide is not present in fresh potatoes, it is formed when the amino acid asparagine and the reducing sugars glucose and fructose are heated at high temperatures. Therefore, lowering the concentrations of free asparagine, glucose, and fructose reduces the acrylamide potential of cooked potatoes.

To determine the effect of lower asparagine and lower reducing sugars in W8, X17, and Y9, acrylamide levels were measured in:

- Fries made from field-grown tubers of W8 and X17 at harvest and after storage at 8 °C and 3 °C; and
- Chips made from field-grown tubers of Y9 at harvest and after storage at 10 °C and 3 °C.

The significantly lower levels of free asparagine and reducing sugars resulted in lower acrylamide potential in W8, X17, and Y9.

Fry Acrylamide Potential of W8

At the time of harvest, fries made with W8 tubers contained 85% less acrylamide than fries made with the Russet Burbank control. When potatoes were stored for three, six, and nine months at 8 °C, acrylamide concentrations in fries made from W8 were also significantly lower than the control (Table 52). When potatoes were stored for six months at 3 °C, acrylamide concentrations in W8 were 86.2% lower than the control and were 90.8% lower after nine months (Table 53).

Table 52. Fry Acrylamide Levels in W8 at Harvest and after Storage at 8 °C

Timing	Variety	Mean (ppb)	P-Value[1]	Standard Deviation	N	Percent Reduction	Range	
							Min	Max
Fresh	W8	75.3	**<.0001**	35.6	41	85.0	32.7	185
	Russet Burbank	503		168	41		229	971
3 Months Storage	W8	86.1	**<.0001**	6.05	9	80.9	74.5	94.3
	Russet Burbank	450		45.7	9		393	514
6 Months Storage	W8	68.3	**0.0011**	19.1	9	83.7	50.4	96.2
	Russet Burbank	420		78.3	9		330	528
9 Months Storage	W8	115	**0.0013**	26.7	9	78.2	90.7	156
	Russet Burbank	528		112	9		429	740

[1]P-values indicating significant differences with controls are underlined and in bold.

Table 53. Fry Acrylamide Levels in W8 after Cold Storage at 3 °C

Timing	Variety	Mean (ppb)	P-Value[1]	Standard Deviation	N	Percent Reduction	Range	
							Min	Max
6 Months Storage	W8	203	**<.0001**	4.04	3	86.2	199	207
	Russet Burbank	1,473		25.2	3		1,450	1,500
9 Months Storage	W8	212	**<.0001**	18.8	3	90.8	201	234
	Russet Burbank	2,300		122	3		2,160	2,380

[1]P-values indicating significant differences with controls are underlined and in bold.

Fry Acrylamide Potential of X17

At the time of harvest, fries made with X17 tubers contained 86% less acrylamide than fries made with the Ranger Russet control (Table 54). When potatoes were stored for six months at 8 °C, acrylamide concentrations were 84.1% lower in X17 fries than the control. When potatoes were stored for six months at 3 °C, acrylamide concentrations in X17 were 93.3% lower than the control (Table 55).

Table 54. Fry Acrylamide Levels in X17 at Harvest and after Storage at 8 °C

Variable	Variety	Mean (ppb)	P-Value[1]	Standard Deviation	N	Percent Reduction	Range	
							Min	Max
Fresh	X17	67.4	**<.0001**	29.9	32	86.1	25.7	113
	Ranger Russet	485		77.0	32		366	650
6 Months Storage[2]	X17	97.6	**<.0001**	35.1	28	84.1	57.9	189
	Ranger Russet	614		199	28		291	1,030

[1]P-values indicating significant differences with controls are underlined and in bold.
[2]6 month storage data not collected from the Adams County, Wisconsin site in 2013.

Table 55. Fry Acrylamide Levels in X17 after Cold Storage at 3 °C

Timing[1]	Variety	Mean (ppb)	P-Value[2]	Standard Deviation	N	Percent Reduction	Range	
							Min	Max
6 Months Storage	X17	170	**<.0001**	7.41	4	93.3	164	180
	Ranger Russet	2,538		86.6	4		2,460	2,660

[1]Data collected from the Minidoka County, Idaho site in 2013.
[2]P-values indicating significant differences with controls are underlined and in bold.

Chip Acrylamide Potential of Y9

At the time of harvest, chips made with Y9 tubers contained 85.1% less acrylamide than chips made with the Atlantic control (Table 56). When potatoes were stored for six months at 10 °C, acrylamide concentrations were 89.0% lower in Y9 chips than the control. When potatoes were stored for six months at 3 °C, acrylamide concentrations in Y9 were 96.8% lower than the control (Table 57).

Table 56. Chip Acrylamide Levels in Y9 at Harvest and after Storage at 10 °C

Timing	Variety	Mean (ppb)	P-Value[1]	Standard Deviation	N	Percent Reduction	Range	
							Min	Max
Fresh	Y9	161	**<.0001**	51.546	28	85.1	112	311
	Atlantic	1,087		499.519	27		397	2,620
6 Months Storage	Y9	175	**<.0001**	48.657	28	89.0	115	266
	Atlantic	1,591		768.366	28		669	3,450

[1]P-values indicating significant differences with controls are underlined and in bold.

Table 57. Chip Acrylamide Levels in Y9 after Cold Storage at 3 °C

Variable[1]	Variety	Mean (ppb)	P-Value[2]	Standard Deviation	N	Percent Reduction	Range	
							Min	Max
6 Months Storage	Y9	541	**<.0001**	175	4	96.8	402	783
	Atlantic	17,100		1,930	4		15,400	19,700

[1]Data collected from the Grant County, Washington site in 2014.
[2]P-values indicating significant differences with controls are underlined and in bold.

Summary of Acrylamide Potential in W8, X17 and Y9

The significantly lower levels of free asparagine and reducing sugars resulted in lower acrylamide potential in W8, X17, and Y9. Although the down regulation of *R1* and *PhL* was less effective, the intended trait of lower reducing sugars is still prevalent in these events from the down regulation of *VInv*. Similar reductions in reducing sugars and acrylamide due to the down regulation of *VInv* were reported by Zhu et al., 2014.

C. Information related to the nutritional impact of the genetically-modified food

Potato has a long history of safe use. Global production in 2013[4] was in excess of 374 million tonnes. Two thirds were consumed directly by humans and the remaining fed to animals or used to produce starch.

The W8, X17, and Y9 events in this submission have been transformed with T-DNA designed to down-regulate endogenous potato genes and to produce the VNT1 protein. The introduction of the RNAi sequences and the VNT1 protein have no nutritional impact on the potato events. This is supported by the fact that:

- Molecular characterisation demonstrated stability of the inserts during vegetative propagation cycles
- The VNT1 protein has homologues with a history of safe consumption and no significant homology to known allergens and toxins; and
- Compositional analysis did not indicate biologically significant changes to the levels of nutrients in events compared to their conventional counterparts. Event composition is within the normal variation of potato cultivars and varieties and is substantially equivalent to conventional potato varieties.

The most important nutritional changes between W8, X17, and Y9 potatoes and their untransformed controls, relate to reduction of the amino acid asparagine (ASN) and reducing sugars, as well as the expression of a late blight resistance protein VNT1. Thus, food products derived from W8, X17, and Y9 potatoes are anticipated to be nutritionally equivalent to food products derived from other commercially available potatoes, except that W8, X17, and Y9 potatoes cooked at high temperatures are expected to have lower acrylamide.

D. Other Information

Where a biotech food has been shown to be compositionally equivalent to conventional varieties, the evidence to date indicates that feeding studies will add little to the safety assessment and generally are not warranted (see e.g. (Bartholomaeus et al., 2013; OECD, 2003; Herman and Ekmay, 2014).

The only new polypeptide produced by the inserts in potato events W8, X17, and Y9 is VNT1. This protein has a non-toxic mode of action and occurs at very low levels in the plants. Its safety is supported by a weight-of-evidence that indicates safety for human consumption. Considering the compositional equivalence between each potato event and its conventional variety, and the lack of any observed phenotypic characteristics indicative of unintended effects arising from the genetic modification process, there was no plausible risk hypothesis that would indicate the need for animal feeding studies.

The regulatory agencies in the United States and Canada have not required feeding studies for events W8, X17, or Y9.

[4] Food and Agriculture Organization of the United Nations, http://faostat3.fao.org; data retrieved 15th January 2016.

Reports

13-04-SPS-ENV 2013 Field Efficacy of W8, X17, and Y9 against *Phytophthora infestans* (Late Blight)

14-04-SPS-ENV 2014 Field Efficacy of W8, X17, and Y9 against *Phytophthora infestans* (Late Blight)

15-04-SPS-MOL Characterization of the Insertion Site in Ranger Russet X17

15-06-SPS-MOL Stability of the DNA Inserts in Ranger Russet X17

15-09-SPS-MOL Expression of *Rpi-vnt1* in X17 Tuber and Leaf Tissues

15-12-SPS-MOL Characterization of the Insertion Sites in Atlantic Y9

15-14-SPS-MOL Stability of DNA Inserts in Atlantic Y9

15-17-SPS-MOL Expression of *Rpi-vnt1* in Y9 Tuber and Leaf Tissues

15-37-SPS-MOL Stability of the DNA Inserts in Russet Burbank W8

15-47-SPS-COMP-RPT Compositional Assessment of W8 Compared to Russet Burbank

15-51-SPS-COMP-RPT Compositional Assessment of X17 Compared to Ranger Russet

15-61-SPS-COMP-02 Compositional Assessment of F10 Compared to Ranger Russet

15-62-SPS-COMP-02 Compositional Assessment of J3 Compared to Atlantic

15-67-SPS-MOL Characterization of Insertion Sites in Russet Burbank W8

15-70-SPS-MOL Expression of *Rpi-vnt1* in W8 Tuber and Leaf Tissues

15-85-SPS-MOL Efficacy of Polyphenol Oxidase Downregulation in W8 Tubers

15-95-SPS-MOL Efficacy of Polyphenol Oxidase Downregulation in X17 Tubers

15-96-SPS-MOL Efficacy of Polyphenol Oxidase Downregulation in Y9 Tubers

15-112-SPS-COMP-RPT Compositional Assessment of Y9 Compared to Atlantic

16-47-SPS-MOL Allergen and Toxin Evaluation of Open Reading Frames in Russet Burbank W8

16-48-SPS-MOL Allergen and Toxin Evaluation of Open Reading Frames in Ranger Russet X17

16-49-SPS-MOL Allergen and Toxin Evaluation of Open Reading Frames in Atlantic Y9

16-72-SPS-ENV 2014 Field Efficacy of W8, X17, and Y9 against *Phytophora infestans* (Late Blight) Strain US-24

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