**FACT SHEET**

**APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF EVENT SPS-ØØØY9-7 (Y9) POTATO FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET**

**NBB REF NO:** JBK (S) 602-1/1/44

The objective of the Biosafety Act is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by SPS International Inc.

## 1. What is the application for?

The application is for import and release of Y9 potato and its products for supply or offer to supply for sale or placing in the market. Y9 potato was developed to address the needs of the potato industry and consumers for potatoes with late blight protection, lower acrylamide potential, reduced black spot, and lower reducing sugars.

## 2. What is the purpose of the import and release?

. The aim of the import is to supply or offer to supply for sale/placing on the market – for direct use as food, feed and processing (FFP) of Y9 potato and its products. The said potato event is not intended for cultivation in Malaysia.

## 3. How has Y9 potato been modified?

Using standard Agrobacterium-mediated transformation, the conventional Atlantic potato variety was transformed with the genetic construct pSIM1278 and pSIM1678 to create the Y9 event. No selectable markers, such as antibiotic or herbicide markers, were used.

The insert from pSIM1278 down regulates polyphenol oxidase, asparagine synthetase, and phosphorylase L transcripts in the potato plant using RNA interference from potato. The insert from pSIM1678 down regulates vacuolar invertase transcripts using RNA interference, and also contains the late blight resistance gene. These inserts are also from wild and cultivated potatoes.

Lower polyphenol oxidase in Y9 potato results in reduced black spot, which improves potato quality and reduces waste. Lower asparagine synthetase, phosphorylase L, and vacuolar contribute to lower free asparagine and reducing sugars, which in turn result in lower acrylamide levels in cooked potatoes. Expression of the late blight resistance gene results in protection against foliar late blight disease, which caused the Irish potato famine. Late blight protection in Y9 enables fewer fungicide applications.

## 4. Characteristics of Y9 potato

### a) Details of the parent organism

The scientific name for cultivated potato is *Solanum tuberosum*. Potato is classified in the *tuberosum* subspecies, within the *tuberosa* series, and within the *potatoe* subsection of the Solanaceae plant family. Plants in the *tuberosum* subspecies are cultivated worldwide.

Potatoes have a long history of safe use as food and feed. They are the world's fourth-largest food crop. Potatoes originated in the Andes region of South America approximately four centuries ago and are now an integral part of the world's food supply.

**b) Details of the donor organism**

The donor organisms are wild and cultivated potato. The sequences transferred into Y9 potato are from *S. tuberosum, Solanum verrucosum,* and *Solanum venturii*. *S. tuberosum* is cultivated potato. *S. verrucosum* and *S. venturii* are used in potato breeding programs and have provided genes for new potato varieties.

**c) Description of the trait(s) and characteristics which have been introduced or modified**

Y9 potatoes have lower free asparagine, lower reducing sugars, lower polyphenol oxidase, and late blight protection. Table 1 summarizes the traits and characteristics that have been introduced or modified in Y9 potato.

**Table 1. Summary of Y9: Genes, Traits, and Benefits**

| Construct | Gene Target / Gene | Mechanism | Trait | Benefit |
|---|---|---|---|---|
| pSIM1278 | *Asn1*: asparagine synthetase-1 | RNAi down regulation | Reduces free asparagine | Contributes to low acrylamide potential[1] |
| | *R1*: water dikinase | | Lowers reducing sugars | |
| | *PhL*: phosphorylase L | | | |
| | *Ppo5*: polyphenol oxidase-5 | | Lowers polyphenol oxidase | Reduces black spot, which improves potato quality and reduces waste |
| pSIM1678 | *Vlnv*: vacuolar invertase | RNAi down regulation | Lowers reducing sugars | Contributes to low acrylamide potential[1] |
| | *Rpi-vnt1*: R-gene | Protein expression | Confers protection against certain strains of *P. infestans* | Late blight protection |

[1]Acrylamide is formed primarily from free asparagine and reducing sugars heated at temperatures above 120 °C, such as during frying of potatoes. According to some global regulators, acrylamide presents a potential health risk for consumers.

**5. Modification Method**

**a) Characteristics of the modification**

Potato variety Atlantic was first transformed with pSIM1278, followed by a subsequent transformation with pSIM1678 to generate the Y9 event.

Plasmid pSIM1278 T-DNA contains two cassettes:

- The first cassette was designed to down regulate asparagine synthetase and polyphenol oxidase transcripts in tubers of transformed potatoes using sequences from *Asn1* and *Ppo5,* respectively. The inverted repeat is comprised of *Asn1* and *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 active in tubers.
- The second cassette was designed to down regulate phosphorylase L and water dikinase transcripts in tubers using sequences from *PhL* and *R1*. The inverted repeat is comprised of *PhL* and *R1* fragments, separated by a spacer element (Spacer-2)

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 active in tubers.

Plasmid pSIM1678 T-DNA contains two cassettes:

- The first cassette contains the 2,676 bp *Rpi-vnt1* (*Vnt1*) gene from *Solanum venturii*. The gene product, VNT1, is a resistance protein (R-protein) involved in the plant immune response that protects potato from late blight infection caused by *P. infestans*. The gene is expressed under the native *Rpi-vnt1* promoter and terminator, pVnt1 and tVnt1, respectively; and
- The second cassette is designed to down regulate vacuolar invertase in tubers of the transformed potato using sequences from vacuolar invertase (*VInv*). The inverted repeat is comprised of VInv fragments, separated by a spacer element (also a VInv fragment) and arranged between two convergent potato promoters: the Agp promoter and the Gbss promoter. Both promoters are active in tubers.

All genetic elements between the left border (LB) and right border (RB) of the two T-DNA originated from cultivated or wild potato species. Figure 1 shows the pSIM1278 and pSIM1678 T-DNA and the resulting structure of the two inserts in Y9 potato.

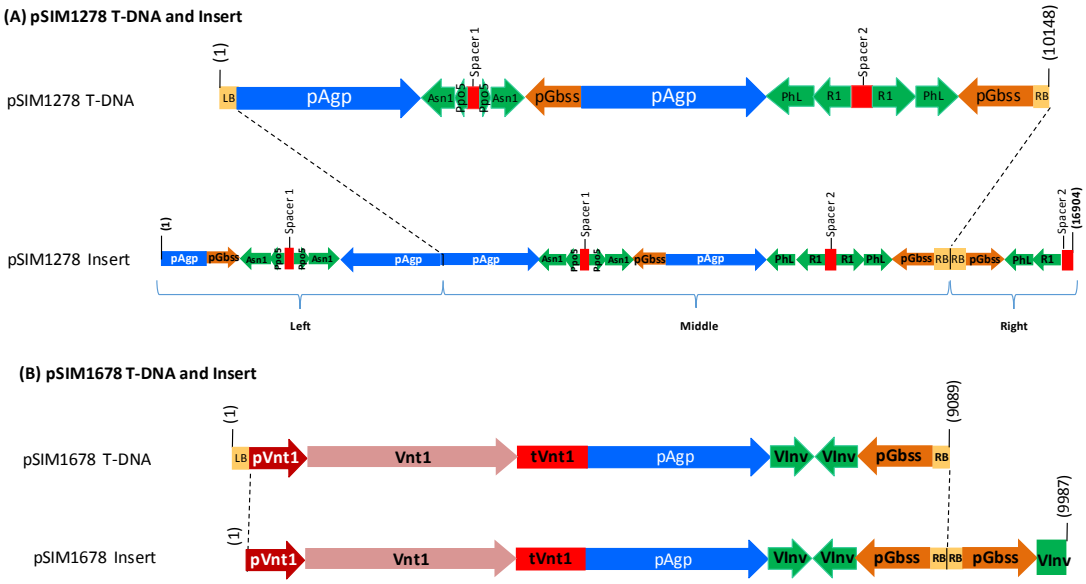


**Diagram of pSIM1278 and pSIM1678 T-DNA and Resulting Inserts in Y9**
(A) The pSIM1278 insert consists of a nearly full-length pSIM1278 T-DNA (Middle) flanked by an additional Asn1/Ppo5 down-regulation cassette on the left side (Left) and a partial PhL/R1 cassette on the right side (Right). (B) The pSIM1678 insert consists of a nearly full-length T-DNA flanked by a partial VInv cassette on the right side.

### b) Safety of the expressed protein

The only expressed protein in Y9 potato is VNT1, encoded by the R-gene *Rpi-vnt1*. An assessment of the potential hazard and potential exposure of VNT1 demonstrated that Y9 potatoes are as safe as conventional potatoes for human and animal consumption.

The *Rpi-vnt1* gene is from wild potato *Solanum venturii*, which is sexually compatible with cultivated potato *S. tuberosum* and used for potato breeding. The VNT1 protein encoded by *Rpi-vnt1* is 98% identical to the protein encoded by *Rpi-vnt1.3*, which is present in a popular European variety, Alouette. VNT1 belongs to a large family of similar proteins found throughout the plant kingdom. Within potato, there are hundreds to thousands of R-proteins

with a long history of safe use. Bioinformatic analysis confirmed that VNT1 lacks sequence homology to known or putative allergens and toxins. Like other R-proteins, VNT1 does not have a toxic mode of action. Rather, it is involved in a hypersensitive response to protect plants through programmed cell death and prevent *P. infestans* spread.

With respect to potential human exposure to VNT1 from consumption of Y9 potatoes, R-genes are tightly regulated and expressed at extremely low levels. Although the *Rpi-vnt1* transcripts were detected by RT-qPCR in Y9 potato tubers at low levels, VNT1 protein levels were too low to detect in Y9 potato tubers (i.e. below limit of quantification). Exposure estimates for humans and livestock showed negligible VNT1 consumption potential from Y9 potato tubers, even using conservative (high-end) assumptions.

## 6. Assessment of Risks to Human Health

### a) Nutritional Data

Compositional analysis of tubers of Y9, Atlantic, and conventional potato varieties were performed to compare nutritional and anti-nutritional analytes and determine ifany nutritionally relevant differences existed between Y9 and conventional potatoes. Y9 potato was found to be substantially equivalent to conventional potatoes.

### b) Toxicology

Glycoalkaloids are toxins commonly found in solanaceous crops, including potato. Together, $\sigma$-solanine and $\sigma$-chaconine make up 95% of the total glycoalkaloids in potato tubers. The widely accepted safety limit for total glycoalkaloids in tubers for human consumption is 20 mg/100 g fresh weight. Levels of glycoalkaloids in Y9 potato were below this limit.

A bioinformatics analysis used to compare open reading frames (ORFs), including the VNT1 protein sequence, generated from the presence of the inserts in Y9 potato against the NCBI database of accessions labeled as “toxin” did not identify any matches or safety concerns.

### c) Allergenicity

Potatoes are not among the “Big Eight” group of foods that account for about 90% of all food allergies in the United States. Patatin (Sol t 1) has been identified as the primary allergen associated with potato. Because potato protein naturally contains a relatively large proportion of patatin, any change in patatin levels in Y9 would be unlikely to affect allergenicity enough to alter consumption patterns for people allergic to potatoes. In addition, patatin levels vary considerably between commonly consumed potato varieties.

A bioinformatic analysis of ORFs, including the VNT1 protein sequence, was conducted using the 2017 AllergenOnline.org database available through the Food Allergy Research and Resource Program via the University of Nebraska. The searches were conducted to identify matches between the protein query sequences and known allergens. None of the searches identified any significant homology.

### d) Safety of Small Interfering RNA

The inserts from pSIM1278 and pSIM1678 produce small interfering RNA that catalyze the degradation of specific mRNA to down regulate target transcripts within the plant. Since siRNA are the product of the transformation, their safety was assessed. This assessment indicated that there is a long history of safe consumption of small RNA. Inserts designed to down regulate host genes for quality traits do not present an increased risk to consumers when compared to currently cultivated food crops.

## 7. Assessment of Risks to the Environment

This application does not cover an environment release (cultivation) of Y9 potato in Malaysia. The release is intended only to cover the import of Y9 potato and its products that may enter Malaysia as foodstuff, feed or for further food processing.
In general, *Solanum tuberosum* is not considered a noxious weed, or reported as a pest or weed in managed ecosystems. It also is not recorded as being invasive of natural ecosystems. Potatoes are known as poor competitors that do not thrive in non-cultivated environments. Safety assessments have not identified any adverse environmental effects related to release of Y9 potato.

## 8. What is the Emergency Response Plan?

### a) First Aid Measures

No special first aid measures are necessary. Safety assessments by the United States USDA, FDA, EPA, the Canadian Food Inspection Agency and Health Canada, and Food Standards Australia New Zealand have shown Y9 to be as safe as other conventional potato varieties.

### b) Accidental Release Measures

Under the Simplot Closed Loop Stewardship Program, Y9 potato tubers are not exported to markets where they are not approved. Any potato tubers imported into Malaysia would need to fulfill quarantine requirements and can be tested for presence of Y9 potato.

SPSII's Closed Loop Stewardship Program details the steps to be taken should an accidental release occur. Response to unintended release would be carried out in consultation with the Malaysian regulatory authorities.

### c) Handling and Storage

Down regulation of invertase can enable Y9 potato tubers to be stored at colder temperatures or for longer periods of time than Atlantic tubers. Simplot is developing guidelines for growers with specific recommendations for storage of Y9 tubers. Although Y9 potatoes have less black spot and bruising because of PPO down regulation, care should still be taken to minimize bruising when handling.

Products such as chips made from Y9 potatoes are stored and handled in the same way as products made from conventional potatoes.

### d) Disposal Considerations

Y9 potato is disposed of in the same ways as conventional potato varieties.

## 9. How can I comment on this application?

Any member of the public may submit their comments or queries on publicly notified information about the application. Before submission of comments or queries, the person should review the information provided. Comments and queries on any possible impacts/risks to the health and safety of the people and the environment that may be posed by the proposed release are appreciated. The submission of comments or queries should be prepared carefully as it will be given the same scrutiny as the application by the NBB. The submission of comments and clarifications or queries should contribute to the NBB's assessment. Even if the submission is not science-based, and focuses on cultural or other values, it should still be developed in the form of a well-founded argument.

Please note that the consultation period closes on 24th June 2018 and written submissions are required by that date. Submissions must be addressed to:

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Please include your full name, address and contact details in your submission.