



# Food safety evaluation for R-proteins introduced by biotechnology: A case study of VNT1 in late blight protected potatoes

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## ABSTRACT

Resistance genes (R-genes) from wild potato species confer protection against disease and can be introduced into cultivated potato varieties using breeding or biotechnology. The R-gene, *Rpi-vnt1*, which encodes the VNT1 protein, protects against late blight, caused by *Phytophthora infestans*. Heterologous expression and purification of active VNT1 in quantities sufficient for regulatory biosafety studies was problematic, making it impractical to generate hazard characterization data. As a case study for R-proteins, a weight-of-evidence, tiered approach was used to evaluate the safety of VNT1. The hazard potential of VNT1 was identified from relevant safety information including history of safe use, bioinformatics, mode of action, expression levels, and dietary intake. From the assessment it was concluded that Tier II hazard characterization was not needed. R-proteins homologous to VNT1 and identified in edible crops, have a history of safe consumption. VNT1 does not share sequence identity with known allergens. Expression levels of R-proteins are generally low, and VNT1 was not detected in potato varieties expressing the *Rpi-vnt1* gene. With minimal hazard and negligible exposure, the risks associated with consumption of R-proteins in late blight protected potatoes are exceedingly low. R-proteins introduced into potatoes to confer late blight protection are safe for consumption.

## 1. Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important food crop after corn, rice, and wheat (FAO, 2008). Late blight caused by the oomycete pathogen, *Phytophthora infestans* (*P. infestans*), is economically one of the most important diseases of potato causing up to \$7.4 billion in annual economic losses (Haverkort et al., 2008). Frequent applications of preventive fungicides are used to manage late blight outbreaks, which increases production costs and exposes growers and the environment to chemical fungicides. R-genes in wild and cultivated *Solanum* species provide natural protection against late blight. R-genes encode R-proteins, which are a natural component of the plant immune response. In the presence of a specific pathogen, R-proteins elicit a hypersensitive response in plants, restricting the spread of the pathogen. Potato varieties containing R-genes effective against disease are often used for breeding into commercial potato varieties to enhance late blight protection. Because commercial potato varieties are tetraploid, highly heterozygous, and sensitive to inbreeding depression, breeding is time consuming and often results in the transfer of undesirable traits (Hirsch et al., 2013). Using biotechnology is advantageous over breeding to efficiently introduce late blight protection into commercial potato varieties.

A comparative safety assessment process has been established and implemented by global regulatory authorities in order to assess the safety of biotech crops (Codex, 2003; FAO/WHO, 2000; OECD, 1993; WHO, 1995). To obtain regulatory approvals for biotech crops to be used as food or feed, a comprehensive assessment is made based on relevant safety data including an evaluation of substantial equivalence to conventional crops through agronomic, phenotypic, and nutrient composition analyses, as well as evaluation of the introduced DNA and the safety of expressed proteins. Over the past 20 years, the majority of biotech crops approved for commercialization have expressed proteins that confer protection against insects or tolerance to herbicides. For protein safety assessment of biotech crops, heterologous systems (e.g. *E. coli*) have typically required sufficient quantities of protein that were evaluated for biochemical, structural, and functional equivalence to the protein produced in the transformed plant.

More recently, biotech crops have been created that express different classes of proteins such as membrane-bound proteins, signal proteins, transcription factors, N-glycosylated proteins, and disease resistance proteins (R-proteins) to achieve traits such as high yield, enhanced nutritional value, drought tolerance, and disease resistance. These proteins are often challenging to produce and purify in sufficient quantities, or with intact biological activity, for use in safety

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evaluations. Bushey et al. (2014) coined “intractable” to describe proteins that are impossible or difficult to express and purify. In 2008, the International Life Sciences Institute (ILSI) and the International Food Biotechnology Committee (IFBiC) made recommendations on a strategy for evaluating the safety of “intractable” proteins using a weight-of-evidence based two-tiered approach. The safety of these proteins can be assessed comprehensively and adequately, without isolation of functional proteins, by considering the weight-of-evidence for Tier I safety assessment, including history of safe use, mode of action, bioinformatics or homology searches, plant protein levels, dietary exposure, and stability assays (Delaney et al., 2008).

Here we conduct a weight-of-evidence based, Tier I safety assessment of an R-protein used to confer late blight protection in potatoes. Based on this assessment it was demonstrated that the risk to human safety (risk = hazard x exposure) of the *Rpi-vnt1* gene in potato was negligible and did not necessitate a Tier II hazard characterization.

## 2. R-genes in plants

### 2.1. R-genes function in plant immunity

Resistance genes (R-genes) are abundant throughout the plant kingdom and make up one of the largest gene families known in plants (Chiang and Coaker, 2015; McHale et al., 2006). Hundreds to thousands of R-genes have been identified that function in the protection of plants against pests and pathogens including bacteria, viruses, fungi, and oomycetes. R-genes encode for R-proteins, which directly or indirectly recognize intracellular avirulence (Avr) effectors that are secreted by pathogens, activating an effector-triggered immunity (ETI) in the host plant (Nimchuk et al., 2003). R-proteins have a non-pesticidal mode of action and do not directly affect pathogens. Instead they activate the plant's immune system, which typically results in a localized plant hypersensitive response (HR), a form of programmed cell death activated at the site of infection. The HR prevents spread of disease by restricting cell-to-cell movement of the pathogen and maintains the overall health of the plant (Gururani et al., 2012).

Most R-proteins consist of nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains, and are often referred to as NBS-LRR proteins. NBS-LRR proteins are divided into four classes: 1) Toll/interleukin-1 receptor (TIR)-NBS-LRR; 2) TIR-TIR-NBS-LRR; 3) coiled-coil (CC)-NBS-LRR; and 4) no obvious motif at N-terminus (X)-NBS-LRR (Fig. 1; McHale et al., 2006). The LRR domain is the least conserved portion of the protein and is involved in protein-protein interactions that determine recognition specificity with pathogen effector proteins (Moffett et al., 2002).

The HR is an active plant immune response and is controlled genetically (Morel and Dangl, 1997). As part of this control, and to avoid spontaneous cell death, R-protein activity is inducible and tightly regulated (Spoel and Dong, 2012). R-protein expression levels are extremely low and an auto-inhibitory mechanism blocks signaling except in the presence of pathogen effectors (Ayliffe et al., 1999; Tan et al., 2007). Regulation of R-protein expression involves transcript synthesis, alternative splicing, transcript degradation, and protein degradation (Marone et al., 2013). There is an evolutionary advantage for a plant to

regulate R-protein expression at low levels, benefiting the plant by conserving metabolic costs and limiting detrimental effects on growth while maintaining effective disease protection. For example, over-expression of an NBS-LRR protein using a constitutive promoter (e.g. 35S) can slow or even block normal growth development. Over-expression of an R-like gene in *Arabidopsis* resulted in a dwarf phenotype (Stokes et al., 2002).

The low expression levels of R-proteins in plants makes it difficult to directly detect R-proteins using current protein detection technology. A literature review identified several publications where researchers attempted to detect or measure the levels of R-proteins in plants (Supplemental Table 1S). In only one case where an R-protein was expressed using its native promoter was it directly detected by immunoblot (Mucyn et al., 2006); in all other cases, the R-protein was not detected in plants unless expressed with affinity tags for detection, expressed under a strong, non-native promoter, or as a truncated version of the full-length protein.

To keep pace with a broad diversity of pathogen species and evolutionary changes to pathogen populations, R-genes in plants have multiplied and evolved. The potato genome contains at least 755 R genes that in some cases share more than 80% sequence identity (Jupé et al., 2013). R genes are mainly found in gene clusters on specific potato chromosomes where higher rates of tandem and segmental gene duplication, recombination, and unequal crossing-over contribute to maintaining an evolutionary arms-race against evolving pathogens (Spoel and Dong, 2012).

### 2.2. R-genes are found in edible crops including potato

R-genes from wild and cultivated food crops exist in the natural environment and food supply. They have a history of safe use and are unlikely to pose a risk to human health, non-target organisms, or the environment. Table 1 shows NBS-LRR R-protein coding genes in edible crops, including potato, demonstrating that R-genes are present in the food supply and have a history of safe consumption.

Potato breeding programs from the mid-twentieth century used the Mexican wild species, *S. demissum*, as a donor for protection against late blight (Pel, 2010; Vleeshouwers et al., 2011). To date, R-genes designated *R1* to *R11* have been identified in *S. demissum*, some of which have been widely used for introgression in European potato breeding programs (Malcolmson and Black, 1966). R-genes have been integrated into many 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). Commercial potato varieties such as Sarpo Mira, Jacqueline Lee, Missaukee, PB-06, Toluca, Bionca, and Alouette all possess R-proteins from wild *Solanum* species that provide protection against late blight (Tables 2 and 3; Vleeshouwers et al., 2011; Vossen et al., 2016). The Alouette potato variety has been used in both conventional and organic production since being introduced into the European market in 2014 (<http://varieties.ahdb.org.uk/varieties/view/Alouette>). The presence of *Rpi-vnt1.3* in Alouette was confirmed by the Plant Research Institute at Wageningen University & Research (Dr. J. Vossen, personal communication). *Rpi-vnt1.3* is a naturally occurring allelic variant of *Rpi-vnt1.1* (Pel et al., 2009). The protein encoded by *Rpi-vnt1.3* is 98% identical to the VNT1 protein encoded by *Rpi-vnt1.1*. Safe consumption of Alouette supports the food safety assessment for the VNT1 protein. The safe consumption of these conventional commercial potatoes demonstrates that R-proteins from wild potato species have a history of safe use in food.

## 3. Tier I safety assessment of the r-protein VNT1: a case study

### 3.1. VNT1 hazard assessment

The *Rpi-vnt1* gene from *S. venturii* (wild potato) was jointly

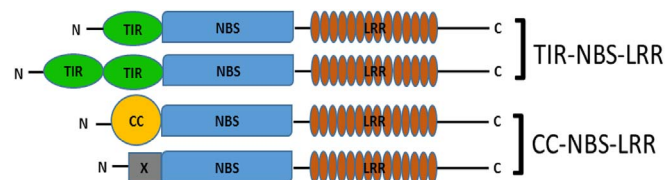


Fig. 1. Classes of NBS-LRR R-proteins: TIR-NBS-LRR and CC-NBS-LRR.

N, amino terminus; TIR, Toll/interleukin-1 receptor-like domain; CC, coiled-coil domain; X, domain without obvious CC motif; NBS, nucleotide binding site; LRR, leucine-rich repeat domain; C, carboxyl terminus. Modified from McHale et al. (2006).

**Table 1**  
Genes Encoding R-Proteins with NBS-LRR Domains are Abundant in Edible Crops.

| Crop                                     | Protein Coding Genes |                                                         | NBS-LRR Genes    |                            | NBS-LRR Genes/Protein Coding Genes (%) |
|------------------------------------------|----------------------|---------------------------------------------------------|------------------|----------------------------|----------------------------------------|
|                                          | Number               | Reference                                               | Number           | Reference                  |                                        |
| Potato ( <i>S. tuberosum</i> )           | 39,031               | Potato Genome Sequencing Consortium, 2011               | 1185             | Wei et al., 2016           | 3.0%                                   |
|                                          |                      |                                                         | 755              | (Jupe et al., 2013)        | 1.9%                                   |
| Tomato ( <i>S. lycopersicum</i> )        | 22,988               | Tomato Genome Consortium, 2012                          | 394              | Jupe et al., 2013          | 1.7%                                   |
| Apple ( <i>Malus x domestica Borkh</i> ) | 57,386               | Velasco et al., 2010                                    | 900              | Velasco et al., 2010       | 1.6%                                   |
| Grape ( <i>Vitis vinifera</i> )          | 30,434               | Jaillon et al., 2007                                    | 459              | Marone et al., 2013        | 1.5%                                   |
| Lettuce ( <i>Lactuca sativa</i> )        | 35,000               | Damerum et al., 2015                                    | 385              | Christopoulou et al., 2015 | 1.1%                                   |
| Spinach ( <i>Spinacia oleracea</i> )     | 25,495               | Xu et al., 2017                                         | 139              | Xu et al., 2017            | 0.5%                                   |
| Rice ( <i>Oryza sativa</i> )             | 37,544               | International Rice Genome Sequencing Project, 2005      | 400              | Monosi et al., 2004        | 1.1%                                   |
| Soybean ( <i>Glycine max</i> )           | 46,430               | Schmutz et al., 2010                                    | 319              | Kang et al., 2012          | 0.7%                                   |
| Barley ( <i>Hordeum vulgare</i> )        | 26,159               | International Barley Genome Sequencing Consortium, 2012 | 175 <sup>a</sup> | Andersen et al., 2016      | 0.7%                                   |
| Maize ( <i>Zea mays</i> )                | 32,000               | Schnable et al., 2009                                   | 150              | Song et al., 2015          | 0.5%                                   |

<sup>a</sup> CC-NBS-LRR only.

**Table 2**  
R-genes in conventional commercial potato varieties that confer late blight protection.

| Growing Region | Commercial Potato Variety (R-gene if known)                                        | Reference                                               |
|----------------|------------------------------------------------------------------------------------|---------------------------------------------------------|
| North America  | Dakota Trailblazer                                                                 | NDSU, 2017                                              |
|                | Defender                                                                           | Novy et al., 2006                                       |
|                | Jacqueline Lee (R8)                                                                | Vossen et al., 2016                                     |
|                | Kennebec (R1)                                                                      | PAA, 2017                                               |
|                | Missaukee (R8)                                                                     | Vossen et al., 2016                                     |
|                | Payette Russet                                                                     | Novy et al., 2017                                       |
| Europe         | Palisade Russet                                                                    | Novy et al., 2012                                       |
|                | Alouette ( <i>Rpi-vnt1.3</i> )                                                     | AHDB, 2017; Dr. J. Vossen, personal communication, 2017 |
|                | Bionica ( <i>Rpi-blb2</i> , <i>Rpi-blb3</i> )                                      | Haverkort et al., 2009; VIB, 2017                       |
|                | Carolus ( <i>Rpi-cha1</i> )                                                        | Haverkort et al., 2016                                  |
|                | Sarpo Mira ( <i>R3a</i> , <i>R3b</i> , <i>R4</i> , <i>R8</i> , <i>Rpi-Smira1</i> ) | Rietman et al., 2012; Vossen et al., 2016               |
|                | Stirling (R2 homolog)                                                              | The James Hutton Institute, 2017                        |
|                | Toluca ( <i>Rpi-blb2</i> , <i>Rpi-blb3</i> )                                       | Haverkort et al., 2009; VIB, 2017                       |
| China          | C-88, PB-06, S-60 (R8)                                                             | Vossen et al., 2016                                     |
| Japan          | Toyoshiro (R1)                                                                     | Japan Root and Tuber Crops Development Association Inc. |
|                | Konafubuki (R1, R3)                                                                | Foundation, 2012                                        |

discovered by the Sainsbury Laboratory (United Kingdom) and Wageningen University (The Netherlands). Late blight protected potato events were generated containing *Rpi-vnt1* with its native promoter and terminator through *Agrobacterium*-mediated transformation. Events W8, X17, and Y9 were selected, comprehensively characterized, and evaluated for late blight efficacy.

The *Rpi-vnt1.1* allele (accession: FJ423044) of the *Rpi-vnt1* gene, hereafter referred as *Rpi-vnt1*, is one of three alleles identified in *S. venturii*. *Rpi-vnt1.1* is predicted to encode an 891 amino acid R-protein belonging to the CC-NBS-LRR class with an estimated molecular weight of 102 kDa, referred to as VNT1.

The VNT1 risk assessment was conducted by considering hazard and exposure potential. A weight-of-evidence approach, considering all information in a comprehensive manner, was used to assess VNT1 safety. VNT1 has minimal hazard potential, taking into consideration the source of the gene, history of safe use of R-protein homologs in crops, including potatoes, homology searches to allergens and toxins using bioinformatics, and mode of action.

### 3.1.1. Source of the *Rpi-vnt1* gene

*S. venturii* is endemic to north-west Argentina (Correll, 1962). *S. venturii* is sexually compatible with cultivated potato *S. tuberosum* and is

used as a donor for late blight protection in European potato breeding programs (Haverkort et al., 2016). Since tubers are typically small, 5–10 mm in diameter (Hawkes and Hjerting, 1960), they are not known to be used as a food source in modern times.

### 3.1.2. VNT1 is homologous to R-proteins with a history of safe use

Proteins with a history of safe use, or those structurally and functionally related to proteins with a history of safe use, are considered safe to consume (Hammond and Cockburn, 2008). As a component of the safety assessment of VNT1, bioinformatic analyses were conducted to identify amino acid sequence homology between VNT1 and other R-proteins, some of which are found in commercial potato and tomato varieties with a history of safe use.

The VNT1 sequence was aligned to other R-proteins that are found in common food crops, including commercial potato and tomato varieties. VNT1 was similar to these R-proteins with 25%–98% sequence identity (Table 3; Lanfermeijer et al., 2003; Mueller et al., 2005).

VNT1 shares up to 98% sequence identity with other R-proteins. All of these proteins are commonly consumed in cultivated potato, tomato (including the Heinz 1706 variety), and pepper, providing support that VNT1 is safe for consumption.

Southern blot data demonstrate that there are many *Rpi-vnt1* homologs in conventional potatoes (Fig. 2). Total genomic DNA from Russet Burbank, Ranger Russet, and Atlantic potato varieties was digested and probed with the entire coding region of the *Rpi-vnt1* gene (Clark et al., 2014; Pence et al., 2016). Many bands in these samples were detected, due to the large number of *Rpi-vnt1* homologs (Fig. 2). Proteins from these *Rpi-vnt1* homologs in the conventional potato genome have a history of safe use.

### 3.1.3. VNT1 lacks sequence homology to allergens and toxins

R-proteins are not known to be allergenic or toxic. VNT1 was assessed for similarity to allergens and toxins using bioinformatics according to guidelines from Codex (2003) and FAO/WHO (2001). The allergen assessment for VNT1 was performed using the Food Allergy Research and Resource Program (FARRP) database and online tools available through the University of Nebraska (<http://www.allergenonline.org/databasefasta.shtml>). Toxicity searches were performed using the standard BLAST (blastp) interface available at NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) against the non-redundant protein sequence database. No significant sequence homology between the VNT1 protein and known or putative allergens or toxins exists. Thus, the VNT1 protein does not share sequence similarity with proteins known to pose allergenic or toxicological concerns.

### 3.1.4. VNT1 mode of action induces the hypersensitive response in plants

VNT1, like other R-proteins, recognizes specific pathogen-secreted

**Table 3**

VNT1 Sequence identity to r-proteins in cultivated potato, tomato and pepper.

| R-protein                                 | Accession    | % Identity | Commercial Variety (Region)                                                        | Source |
|-------------------------------------------|--------------|------------|------------------------------------------------------------------------------------|--------|
| VNT1.3 ( <i>Rpi-vnt1.3</i> ) <sup>a</sup> | ACJ66596     | 98         | Alouette (Europe)                                                                  | potato |
| RPP13-like protein                        | XP_006360932 | 87         |                                                                                    | potato |
| MCQ1 ( <i>Rpi-mcq1</i> ) <sup>a</sup>     | GNO43561     | 80         |                                                                                    | potato |
| RPP13-like protein 3                      | XP_006360919 | 80         |                                                                                    | potato |
| RPP13-like protein 3                      | XP_015170548 | 79         |                                                                                    | potato |
| RPP-13 like protein                       | XP_004247881 | 77         | Heinz 1706 (North America)                                                         | tomato |
| RPP13-like protein 3                      | XP_006360612 | 77         |                                                                                    | potato |
| St-Tm-2 ToMV                              | ABM05492     | 77         |                                                                                    | potato |
| Ph-3 <sup>a</sup>                         | AIB02970     | 77         | Iron Lady, Plum Regal, Mountain Magic, Mountain Merit, Defiant PHR (North America) | tomato |
| RPP13-like protein                        | XP_015170549 | 76         |                                                                                    | potato |
| Tm-2 ToMV                                 | AAQ10735     | 76         |                                                                                    | tomato |
| Tm-2 <sup>2</sup> ToMV                    | AAQ10736     | 76         |                                                                                    | tomato |
| tm-2                                      | AAQ10734     | 76         |                                                                                    | tomato |
| Tm-2 ToMV                                 | AAV87531     | 75         |                                                                                    | tomato |
| RPP13-like protein 3                      | XP_016566165 | 74         |                                                                                    | pepper |
| BLB2 ( <i>Rpi-blb2</i> ) <sup>a</sup>     | AAZ95005     | 32         | Bionica, Toluca (Europe)                                                           | potato |
| R2 <sup>a</sup>                           | ADL59401     | 32         | Stirling (Europe)                                                                  | potato |
| R1 <sup>a</sup>                           | CAD88974     | 30         | Kennebec (North America), Toyoshiro, Konafubuki (Japan)                            | potato |
| R3a <sup>a</sup>                          | AAW48299     | 29         | Sarpo Mira                                                                         | potato |
| RGA1 <sup>a</sup>                         | Q7XA42       | 25         |                                                                                    | potato |

\*Margaret T. McGrath, Karen LaMarsh, and Sandra Mensasha. Late blight resistant tomato variety evaluation conducted with organic production practices, New York 2013. Midwest Vegetable Trial Report for 2013.

<sup>a</sup> Functional homologs of VNT1, these R-proteins recognize effectors secreted by the late blight pathogen, *P. infestans*.

effector proteins (e.g. Avr-vnt1) from *P. infestans*, and induces the plant hypersensitive response to protect the plant by limiting pathogen spread (Fig. 3; Dodds and Rathjen, 2010; Spoel and Dong, 2012; Stefanczyk et al., 2017). Unlike Bt proteins in some biotech crops, which are toxic to insects when ingested, VNT1 directly affects the plant, not the pathogen.

### 3.2. VNT1 exposure assessment

The consumption of late blight protected potatoes presents negligible exposure to VNT1, taking into consideration the level of VNT1

expression in tubers and the potential dietary exposure of VNT1 in humans and livestock.

#### 3.2.1. Expression of *Rpi-vnt1* transcripts in late blight protected potatoes

Expression of *Rpi-vnt1* transcripts in late blight protected potatoes is regulated by its native promoter and terminator. Northern blots and reverse transcription quantitative PCR (RT-qPCR) was used to measure *Rpi-vnt1* transcript levels in tuber and leaf tissues. The results showed that *Rpi-vnt1* transcripts were detected at low levels, yet were sufficient to confer foliar late blight protection, and are otherwise agronomically equivalent (Clark et al., 2014; Pence et al., 2016). These observations

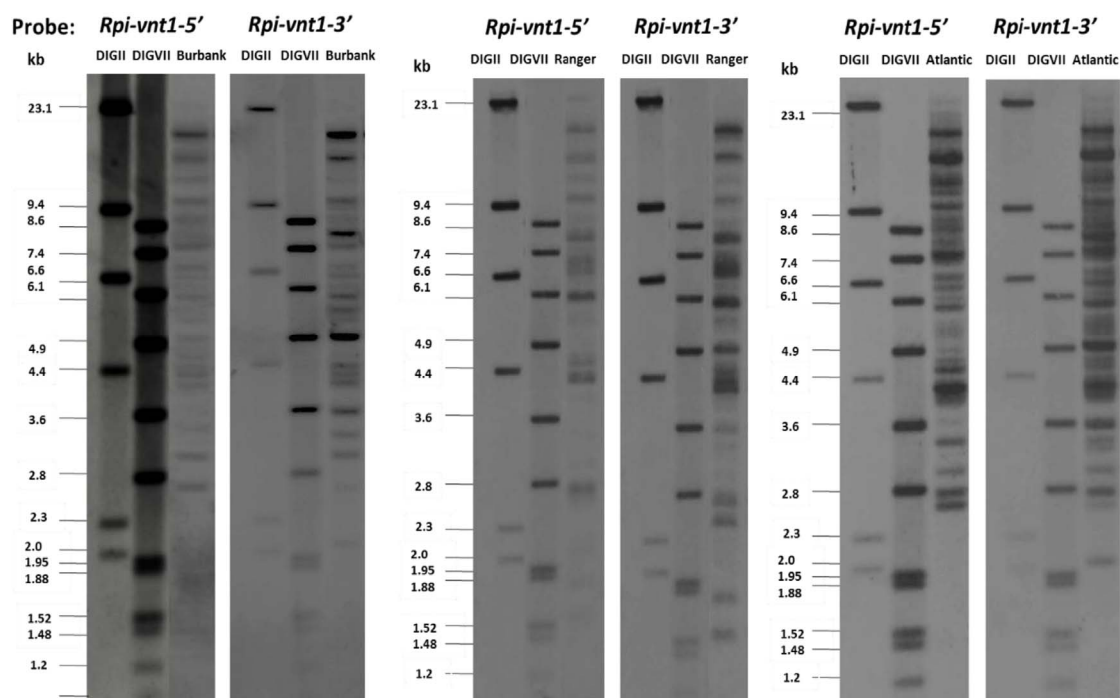


**Fig. 2. *Rpi-vnt1* Homologs are Abundant in the Conventional Commercial Potato Genome.**

Southern blots of *BbsI* digested genomic DNA isolated from Russet Burbank (Burbank), Ranger Russet (Ranger) and Atlantic. Blots were hybridized with the DIG-labeled *Rpi-vnt1* probes (*Rpi-vnt1-5'* and *Rpi-vnt1-3'*, spanning the entire coding region). The molecular weight markers, DIGII and DIGVII, were labeled in kb.

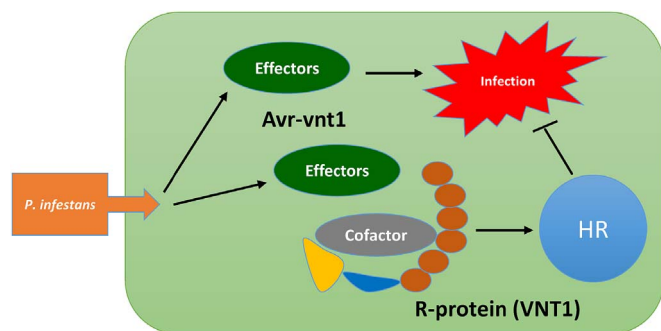


**Fig. 3.** VNT1 Mode of Action Involves the Hypersensitive Response in Plants.

VNT1 directly, or indirectly through a co-factor, interacts with the Avr-vnt1 effector, induces the plant hypersensitive response, and prevents infection by *P. infestans*. Avr-vnt1: *P. infestans* expressed effector; Co-factor: a molecule that mediates the interaction between an effector and R-protein; HR: plant hypersensitive response.

are consistent with scientific literature indicating that R-genes are tightly regulated and expressed at low levels (Marone et al., 2013; Stokes et al., 2002).

### 3.2.2. VNT1 protein detection in late blight protected potato tubers

Similar to other R-proteins, the VNT1 protein is expressed at low levels in plant tissues, making it challenging to detect and quantify in late blight protected potatoes. A liquid chromatography–mass spectrometry (LC-MS) method was developed to quantitate VNT1 in potato tissues. Using isotope labeled peptides unique to VNT1 spiked with protein extracts from tubers, the LOD for VNT1 was established at 500 parts per billion (ppb). The endogenous unlabeled VNT1 peptides were undetected, indicating the concentration of VNT1 in tubers was below 500 ppb (data not shown).

To corroborate the LC-MS results, western blots were used to assess VNT1 levels in late blight protected potato tissues. A polyclonal antibody ( $\alpha$ -VNT1-602) with high sensitivity and specificity was produced against a peptide from the LRR domain of VNT1. An LOD using this antibody was established at less than 500 ppb by a serial dilution of a recombinant VNT1 standard spiked into potato protein extracts. The VNT1 standards (1.25 ng and 2.5 ng) were spiked into protein extracts as positive controls. In three different W8 samples, there were no observable bands between 75 and 150 kDa. Thus, VNT1 (estimated molecular weight of 102 kDa) was not detected in these samples (Fig. 4). Some non-specific cross-reactivity using the  $\alpha$ -VNT1-602 antibody was observed. However, this is not necessarily unexpected as many *Rpi-vnt1* homologs are known to exist in potatoes (demonstrated in Fig. 2) (see Fig. 5).

The western blot and LC-MS results were consistent with one another, showing that the VNT1 protein expressed in late blight protected potatoes was too low to detect (i.e. below LOD). Based on these methods, the level of VNT1 in tubers was established to be less than 500 ppb.

To put VNT1 expression level into perspective, Herculex 1<sup>®</sup> maize (TC1507), Roundup Ready<sup>®</sup> soybean (GTS 40-3-2), and Roundup Ready<sup>®</sup> maize are used for comparison. These biotech crops broadly cultivated worldwide with a history of safe consumption as food and feed. They express the insecticidal protein Cry1F and the herbicidal protein CP4 EPSPS, respectively. The Cry1F protein level in maize grain was 93 parts per million (ppm) (range 71–115 ppm), CP4 EPSPS in soybean grain 290 ppm (range 186–395 ppm) and Roundup Ready<sup>®</sup> maize 10–14 ppm (Hammond and Cockburn, 2008). In comparison, VNT1 level in tubers was estimated at less than 500 ppb, tens to hundreds of times lower than human and livestock exposure to Cry1F in maize and CP4 EPSPS in Roundup Ready<sup>®</sup> soybean and maize.

### 3.2.3. Low dietary exposure of humans and livestock to VNT1

Animals (with the exception of insect pests) are not known to graze

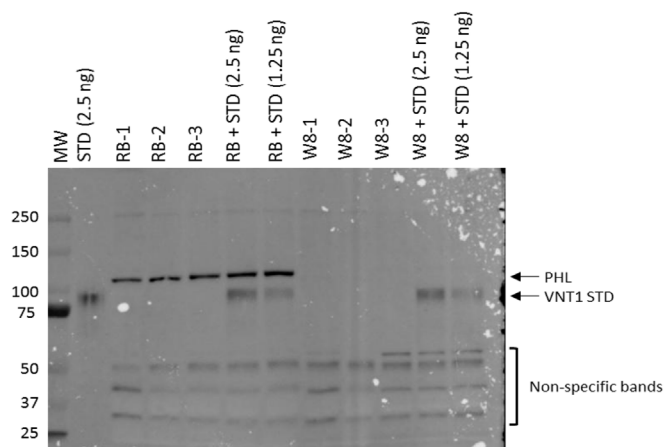


**Fig. 4.** VNT1 was not Detected in W8 Late Blight Protected Potatoes by Western Blot.

VNT1 levels were below detection limits in three biological replicates of W8 tuber samples following western blot analysis using  $\alpha$ -VNT1-602 antibody. Protein extracts from Russet Burbank (RB) and W8 samples were each spiked with two concentrations of VNT1 standard (STD) (1.25 ng and 2.5 ng corresponding to 220 ppb and 500 ppb, respectively). Western blot analysis of RB and W8 samples showed the absence of VNT1 in tuber samples. The antibody had cross-reactivity with PHL protein (estimated molecular weight of 109 kDa), which is down regulated and absent in W8 tubers.

on potato leaves in the field due to the presence of glycoalkaloids in Solanaceae leaves (Kozukue et al., 1987; Maga, 1994). As such, the dietary exposure assessment of humans and livestock to VNT1 focused on tubers. The LOD value of 500 ppb, established by LC-MS and western blot methods, was used to calculate the potential dietary exposure of humans and livestock to VNT1. Given that VNT1 is too low to detect in tubers, dietary exposure is expected to be negligible in humans and livestock.

### 3.2.4. VNT1 exposure assessment in humans

The human exposure calculations used United States potato consumption data from the Dietary Exposure Evaluation Model – Food Commodity Intake Database (DEEM-FCID) (EPA, 2014). United States potato consumption rates are higher than other regions of the world except Europe and include a wide variety of potato sources such as fries, hash browns, chips, dry flakes, and flour present in infant food (FAO, 2008). Although from the United States, these data are applicable to other populations. Acute and chronic potato consumption rates were calculated using DEEM-FCID (Table 4, Supplemental Table 2S). Acute consumption levels represent high consumers, that is, individuals that eat more potatoes and potato-containing products than 95% of the population, whereas the chronic data are average consumption levels. The population subgroup with the highest potato consumption was children aged 1–2 (Supplemental Table 2S).

The acute exposure (i.e. single day) estimate for VNT1 in late blight protected potatoes was 0.0015 mg/kg bw for high consuming individuals within the United States population, and 0.0041 mg/kg bw for children aged 1–2. The chronic (i.e. continual or daily) exposure was estimated at 0.00034 mg/kg bw/d or 0.00086 mg/kg bw/d for an average individual or child aged 1–2, respectively (Table 4).

To our knowledge, the No-Observed-Adverse-Effect Level (NOEL) has not been established for any R-protein. This is likely attributed to the difficulties in extracting or expressing large quantities of these proteins required for toxicological studies and a lack of need to conduct such studies. In the absence of an experimental NOEL for VNT1, the margin of exposure could not be calculated. To put VNT1 exposure levels into perspective, the calculated exposure levels for VNT1 were compared to i) the threshold for toxicological concern (TTC, 0.030 mg/kg bw/day) for small molecules with no structural alerts (Kroes et al., 2004), ii) the acute (17.9 mg/kg bw) and chronic (2.49 mg/kg bw/day)

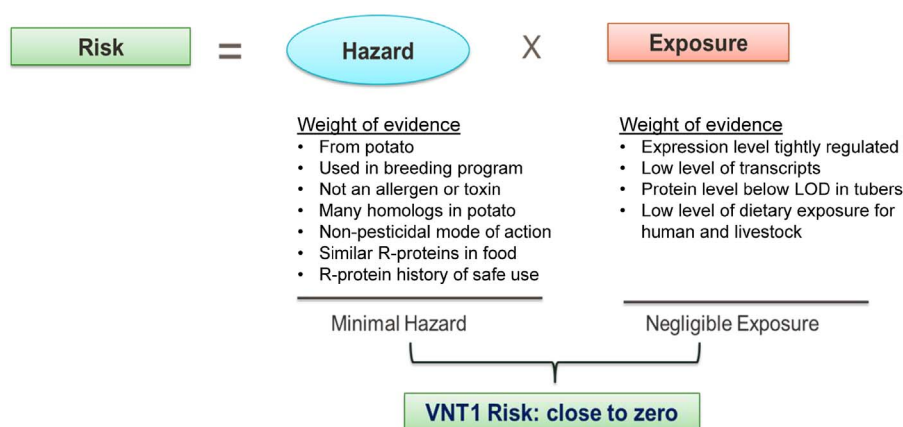


Fig. 5. The Risk from Consuming VNT1 in Late Blight Protected Potatoes is Negligible.

Risk assessment principles, considering potential hazard and exposure, were applied to evaluate risk associated with consumption of VNT1 protein from late blight protected potatoes.

TTC's adapted for use in biotechnology-derived crops (Hammond and Cockburn, 2008), and iii) the consumption of a protein, CP4 EPSPS (0.004 mg/kg bw/d) found in Roundup Ready<sup>®</sup> maize (Hammond et al., 2013).

The threshold of toxicological concern (TTC) concept, established for chemicals, can be used to evaluate VNT1 exposure. According to (Kroes et al., 2004), the TTC “is a pragmatic risk assessment tool that is based on the principle of establishing a human exposure threshold value for chemicals, below which there is a very low probability of an appreciable risk to human health.” The thresholds vary based on the chemical structure, and they range from 1.5 µg/kg bw/day for chemicals with a high level of concern to 30 µg/kg bw/day for chemicals with no structural alerts. The most relevant TTC for a protein would be a chemical with no structural alerts (e.g. 0.03 mg/kg bw/day; (Kroes et al., 2004). Chronic VNT1 consumption (0.00034 mg/kg bw/day) is approximately 90 times lower than the TTC established for chemicals. Therefore, the average adult would need to consume approximately 60 g of potato per kg bw on a daily basis to reach the TTC for a chemical with no structural alerts. Roughly, this translates to eating 4 kg of late-blight protected potatoes each day in order to reach the TTC. This chemical-derived threshold, suggests that consumption of late blight protected potatoes with VNT1 has an adequate safety margin and would not require further Tier II safety testing.

The TTC concept has been applied to proteins introduced using biotechnology (Hammond and Cockburn, 2008). The TTC values relate to a subset of proteins that have no structural relatedness to known allergens or toxins. The acute (17.9 mg/kg) and chronic (2.49 mg/kg bw/day) thresholds were derived from the average NOAEL values, divided by a safety factor (e.g. 100), identified in either 30 acute or 40 subchronic toxicological studies of multiple proteins. Bioinformatic analysis of VNT1 did not reveal structural similarities to allergens or toxins, so is suitable for comparison to protein TTC values. VNT1 exposure is approximately 12,000 times and 7000 times lower than the acute and chronic protein TTC, respectively. Using these comparators, an individual would need to consume approximately 2500 kg of late-

blight protected potatoes in a single day to reach the acute TTC (e.g. 17.9 mg/kg). Alternatively, daily consumption of 350 kg of late blight protected potatoes would be required to reach the chronic TTC value (2.49 mg/kg bw/day). This comparison indicated that VNT1 exposure is minimal and poses negligible risks as with other approved biotech proteins in food.

A comparison was drawn between VNT1 exposure from late blight protected potatoes and CP4 EPSPS exposure from a single biotech crop, Roundup ready<sup>®</sup> maize. CP4 EPSPS is an enzyme that confers resistance to the herbicide glyphosate. The CP4 EPSPS protein was introduced through biotechnology into a variety of crops that are widely consumed by humans and livestock, including alfalfa, canola, cotton, soybean, sugar beet, wheat, potato and maize (<http://www.isaaa.org/gmapprovaldatabase>). An estimate of daily (chronic) consumption of CP4 EPSPS was calculated in maize as 0.004 mg/kg bw/day (Hammond et al., 2013). VNT1 consumption in late blight protected potato (e.g. 0.00034 mg/kg bw/day) is approximately 12 times lower than the CP4 EPSPS in maize, which has a history of safe consumption.

This dietary exposure assessment was an overestimate based on a number of conservative assumptions including: the amount of VNT1 in tubers was assumed to be the limit of detection (500 ppb), which is greater than the actual amount; United States potato consumption data was used, which is higher than other global regions except Europe; potato consumption values of consumers at the 95th percentile were used; 100% of the potatoes consumed in the diet were from late blight protected potatoes; and there was no degradation of VNT1 during cooking and processing. Even with conservative assumptions, the estimated human dietary exposure to VNT1 from late blight protected potatoes remains negligible.

### 3.2.5. VNT1 exposure assessment in livestock

Unwanted potato material is sometimes incorporated into ruminant livestock feed. In the United States, approximately 1% of unwanted potatoes is used as livestock feed (NPC, 2016). This can include potato culls (whole unpeeled potato not suited for fresh market or processing)

Table 4  
Dietary exposure of humans to VNT1 from late blight protected potatoes.

| Population <sup>a</sup> | VNT1 in Potatoes (µg/g fw tubers) <sup>b</sup> | Chronic Potato Consumption g/kg bw/d | Acute Potato Consumption (g/kg bw/d) <sup>c</sup> | Estimated chronic VNT1 Exposure (mg/kg bw/d) <sup>d</sup> | Estimated acute VNT1 Exposure <sup>d</sup> (mg/kg bw/d) |
|-------------------------|------------------------------------------------|--------------------------------------|---------------------------------------------------|-----------------------------------------------------------|---------------------------------------------------------|
| Total U.S. population   | 0.5                                            | 0.68                                 | 3.05                                              | 0.00034                                                   | 0.0015                                                  |
| Children Ages 1-2       | 0.5                                            | 1.72                                 | 8.19                                              | 0.00086                                                   | 0.0041                                                  |

<sup>a</sup> Further details on the DEEM-FCID methodology and data for all sub-populations are available in the supplemental data.

<sup>b</sup> 500 ppb VNT1 in fresh weight tubers = 0.5 µg/g in fresh weight tubers.

<sup>c</sup> Consumption at 95th Percentile.

<sup>d</sup> Exposure to VNT1 = (Potato Consumption) × (0.5 µg VNT1/g tubers) × (0.001 mg/µg).

**Table 5**  
Late blight protected potato events.

| Parental variety | Event | Country Approvals/Deregulations (as 9/25/2017)                         |
|------------------|-------|------------------------------------------------------------------------|
| Russet Burbank   | W8    | U.S. <sup>a,b,c,d</sup> , Canada <sup>e,f</sup> , FSANZ <sup>g</sup>   |
| Ranger Russet    | X17   | U.S. <sup>d,g,h,i,j</sup> , Canada <sup>e,f</sup> , FSANZ <sup>g</sup> |
| Atlantic         | Y9    | U.S. <sup>d,g,h,i,j</sup> , Canada <sup>e,f</sup> , FSANZ <sup>g</sup> |

<sup>a</sup> USDA APHIS: [https://www.aphis.usda.gov/brs/aphisdocs/14\\_09301p\\_det.pdf](https://www.aphis.usda.gov/brs/aphisdocs/14_09301p_det.pdf).

<sup>b</sup> US FDA: <https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/Submissions/ucm486694.htm>.

<sup>c</sup> US EPA: search for 8917-1 at <https://iaspub.epa.gov/apex/pesticides/f?p=PPLS:1>.

<sup>d</sup> US EPA: <https://www.federalregister.gov/documents/2017/02/24/2017-03580/vnt1-protein-in-potato-exemption-from-the-requirement-of-a-tolerance>.

<sup>e</sup> Health Canada: <https://www.canada.ca/en/health-canada/services/food-nutrition/genetically-modified-foods-other-novel-foods/approved-products/simplot-innate-potato.html>.

<sup>f</sup> CFIA: approved but not yet posted on website.

<sup>g</sup> USDA-APHIS: [https://www.aphis.usda.gov/brs/aphisdocs/16\\_06401p\\_det\\_pprs.pdf](https://www.aphis.usda.gov/brs/aphisdocs/16_06401p_det_pprs.pdf).

<sup>h</sup> US FDA: <https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/Submissions/ucm542341>.

<sup>i</sup> US EPA: search for 8917-2 at <https://iaspub.epa.gov/apex/pesticides/f?p=PPLS:1>.

<sup>j</sup> US EPA: search for 8917-3 at <https://iaspub.epa.gov/apex/pesticides/f?p=PPLS:1>.

<sup>k</sup> FSANZ: search for A1139 at <http://www.foodstandards.gov.au/code/changes/gazette/Pages/AmendmentNo175.aspx>.

and potato process waste (wet and dry peel, raw chip, fries, and cooked potatoes).

The potential exposure of livestock to VNT1 from late blight protected potatoes was evaluated by calculating potato consumption based on potato incorporation rates of 6%, 2%, and 30% dry matter into feed for cattle, sheep and pig, respectively (Supplemental Table 3S) multiplied by VNT1 in tubers (e.g. 500 ppb). According to these calculations, pigs had the highest estimated VNT1 exposure from consumption of cooked late blight protected potatoes at 0.023 mg/kg bw/d, which is below the TTC (0.03 mg/kg bw/d) for chemicals with no structural alerts (Kroes et al., 2004). VNT1 exposure estimates in cattle were approximately 10-fold lower than in pigs. Adult sheep were predicted to have the lowest VNT1 exposure at 0.00088 mg/kg bw/day. Similar to the human dietary exposure analysis, livestock have negligible exposure to VNT1 consumed from late blight protected potatoes, even using conservative assumptions.

R-proteins are tightly regulated and expressed at extremely low levels, which provides effective plant protection and at the same time conserves the metabolic cost and limits detrimental effects on plant growth. The *Rpi-vnt1* transcripts were measured in late blight protected potato tissues at very low levels. VNT1 protein levels were below the detection limit. Both humans and livestock have negligible exposure to VNT1 from late blight protected potatoes, even using conservative assumptions. It can be concluded that human and livestock exposure to VNT1 from late blight protected potatoes is negligible.

#### 4. Conclusion: minimal risk associated with VNT1

The safety of VNT1 protein in late blight protected potatoes was evaluated using a weight of evidence approach and was concluded that the additional risk of introduced VNT1 is negligible and late blight protected potatoes are considered to be as safe as conventional varieties lacking VNT1. This conclusion was derived from the following:

- VNT1 is encoded by the *Rpi-vnt1* gene from wild potato *S. venturii*, which is sexually compatible with cultivated potato and used for potato breeding;
- There is a history of safe consumption of R-proteins in edible crops, which have hundreds of R-proteins;
- VNT1 is homologous with R-proteins in conventional potatoes, pepper and tomato;
- VNT1 is not homologous to allergens and toxins;

- VNT1 activates the plant immune response and directly affects the plant, not the pathogen;
- The VNT1 protein is too low to detect in late blight protected tubers; and
- VNT1 has negligible dietary exposure to humans and livestock.

Regulatory agencies, including USDA Animal and Plant Health Inspection Service (USDA-APHIS), US Food and Drug Administration (US FDA), US Environmental Protection Agency (US EPA), Canadian Food Inspection Agency (CFIA), Health Canada, and Food Standards Australia and New Zealand (FSANZ) have reviewed comprehensive safety data packages that included a VNT1 R-protein safety assessment and have approved late blight protected potatoes without isolation of the R-protein (Table 5). After reviewing VNT1 safety information, these agencies concluded that the VNT1 protein in late blight protected potatoes is safe for humans, livestock, other consumers, and the environment, as hundreds of similar R-proteins exist in edible crops. US EPA reviewed the VNT1 safety information and granted a permanent exemption from the requirement of a tolerance for VNT1 protein in late blight protected potatoes stating that “there is a reasonable certainty that no harm will result from aggregate exposure to residues of VNT1 protein” (Federal Register Vol.82, No. 36, 2017).

When both the minimal hazard and the negligible exposure potential are considered together, the potential risk associated with consuming VNT1 in late blight protected potatoes is negligible. These considerations complete a Tier I evaluation of the R-protein, VNT1. The absence of an identified hazard or high exposure indicates that further investigation (e.g. Tier II evaluation) is not warranted.

This assessment method is also applicable to other intractable proteins. Several regulatory agencies including USDA-APHIS, CFIA, FSANZ, and Argentina Biotechnology Directorate (CONABIA), have already adopted approaches to allow for construct-based evaluation of events (Beker et al., 2016; Halterman et al., 2016). As an extension, the conclusion that R-proteins such as VNT1 are safe to consume should be applicable to the same or similar R-proteins expressed in different potato varieties.

#### Conflicts of interest

The authors are employed by J.R. Simplot Company that develops biotech potato crops.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.yrtph.2018.03.008>

#### Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.yrtph.2018.03.008>

#### References

- AHDB, 2017. Alouette. Agriculture and Horticulture Development Board. Potato Variety Database. <http://varieties.ahdb.org.uk/varieties/view/Alouette>.
- Andersen, E.J., Ali, S., Reese, R.N., Yen, Y., Neupane, S., 2016. Diversity and evolution of disease resistance genes in barley (*Hordeum vulgare* L.). *Evolutionary Bioinformatics*

- Online 12, 99–108.
- Ayliffe, M.A., Frost, D.V., Finnegan, E.J., Lawrence, G.J., Anderson, P.A., Ellis, J.G., 1999. Analysis of alternative transcripts of the flax L6 rust resistance gene. *Plant J.* 17, 287–292.
- Beker, M.P., Boari, P., Burachik, M., Cuadrado, V., Junco, M., Lede, S., Lema, M.A., Lewi, D., Maggi, A., Meoniz, I., et al., 2016. Development of a construct-based risk assessment framework for genetic engineered crops. *Transgenic Res.* 25, 597–607.
- Bushey, D.F., Bannon, G.A., Delaney, B.F., Graser, G., Hefford, M., Jiang, X., Lee, T.C., Madduri, K.M., Pariza, M., Privalle, L.S., et al., 2014. Characteristics and safety assessment of intractable proteins in genetically modified crops. *Regul. Toxicol. Pharmacol.* 69, 154–170.
- Chiang, Y.-H., Coaker, G., 2015. Effector Triggered Immunity: NLR Immune Perception and Downstream Defense Responses. *The Arabidopsis Book* 11 e0183.
- Christopoulou, M., Wo, S.R.-C., Kozik, A., McHale, L.K., Truco, M.-J., Wroblewski, T., Micheltore, R.W., 2015. Genome-wide architecture of disease resistance genes in lettuce. *Genes Genomes Genetics* 5, 2655–2669.
- Clark, P., Habig, J., Ye, J., Collinge, S., 2014. United States Department of Agriculture Animal and Plant Health Inspection Service. Petition for Determination of Nonregulated Status for Innate™ Potatoes with Late Blight Resistance, Low Acrylamide Potential, Reduced Black Spot, and Lowered Reducing Sug. USDA APHIS. [https://www.aphis.usda.gov/brs/aphisdocs/14\\_09301p.pdf](https://www.aphis.usda.gov/brs/aphisdocs/14_09301p.pdf).
- Codex, 2003. Guideline for the Conduct of Food Safety Assessment of Foods Derived from the Recombinant-dna Plants. Codex Alimentarius Commission CAC/GL 45-2003, pp. 1–18.
- Correll, D.S., 1962. *Solanum verrucosum*. In: *The Potato and its Wild Relatives*. Texas Research Foundation, pp. 360–364.
- Damerum, A., Selmes, S.L., Biggi, G.F., Clarkson, G.J., Rothwell, S.D., Truco, M.J., Micheltore, R.W., Hancock, R.D., Shellcock, C., Chapman, M.A., et al., 2015. Elucidating the genetic basis of antioxidant status in lettuce (*Lactuca sativa*). *Horticult. Res.* 2, 15055.
- Delaney, B., Astwood, J.D., Cuny, H., Conn, R.E., Herouet-Guicheney, C., Macintosh, S., Meyer, L.S., Privalle, L., Gao, Y., Mattsson, J., et al., 2008. Evaluation of protein safety in the context of agricultural biotechnology. *Food Chem. Toxicol.* 46, S71–S97.
- Dodds, P.N., Rathjen, J.P., 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat. Rev. Genet.* 11, 539–548.
- EPA, 2014. United States Environmental Protection Agency. DEEM-FCID/Calindex Software Installer. <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/deem-fcidcalindex-software-installer>.
- FAO, 2008. International Year of the Potato 2008: New Light on a Hidden Treasure. Food and Agriculture Organization. <http://www.fao.org/potato-2008/en/world/>.
- FAO/WHO, 2000. Safety Aspects of Genetically Modified Foods of Plant Origin. Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology. [http://www.fao.org/fileadmin/templates/agns/pdf/topics/ec\\_june2000\\_en.pdf](http://www.fao.org/fileadmin/templates/agns/pdf/topics/ec_june2000_en.pdf).
- FAO/WHO, 2001. Evaluation of Allergenicity of Genetically Modified Foods. Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. [http://www.who.int/fsf/GMfood/Consultation\\_Jan2001/report20.pdf](http://www.who.int/fsf/GMfood/Consultation_Jan2001/report20.pdf).
- Gururani, M.A., Venkatesh, J., Upadhyaya, C.P., Nookaraju, A., Pandey, S.K., Park, S.W., 2012. Plant disease resistance genes: current status and future directions. *Physiol. Mol. Plant Pathol.* 78, 51–65.
- Haltermann, D., Guenther, J., Collinge, S., Butler, N., Douches, D., 2016. Biotech potatoes in the 21st century: 20 Years since the first biotech potato. *Am. J. Potato Res.* 93, 1–20.
- Hammond, B., Cockburn, A., 2008. The safety assessment of proteins introduced into crops developed through agricultural biotechnology. In: *Food Safety of Proteins in Agricultural Biotechnology*. CRC Press, pp. 259–288.
- Hammond, B., Kough, J., Herouet-Guicheney, C., Jez, J.M., 2013. Toxicological evaluation of proteins introduced into food crops. *Crit. Rev. Toxicol.* 43 (Suppl. 2), 25–42.
- Haverkort, A.J., Boonekamp, P.M., Hutten, R., Jacobsen, E., Lotz, L.A.P., Kessel, G.J.T., Visser, R.G.F., Vossen, E.A.G., 2008. Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. *Potato Res.* 51, 47–57.
- Haverkort, A.J., Struik, P.C., Visser, R.G.F., Jacobsen, E., 2009. Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans*. *Potato Res.* 52, 249–264.
- Haverkort, A.J., Boonekamp, P.M., Hutten, R., Jacobsen, E., Lotz, L.A.P., Kessel, G.J.T., Vossen, J.H., Visser, R.G.F., 2016. Durable late blight resistance in potato through dynamic varieties obtained by cisgenesis: scientific and societal advances in the DuRPh project. *Potato Res.* 59, 35–66.
- Hawkes, J., Hjerting, J., 1960. Some wild potato species from Argentina. *Phyton* 9, 140–146.
- Hirsch, C.N., Hirsch, C.D., Felcher, K., Coombs, J., Zarka, D., Van Deynze, A., De Jong, W., Veilleux, R.E., Jansky, S., Bethke, P., et al., 2013. Retrospective view of north American potato (*Solanum tuberosum* L.) breeding in the 20<sup>th</sup> and 21<sup>st</sup> Centuries. *G3. Genes Genomes Genetics* 3, 1003–1013.
- Hutten, R., van Berloo, R., Finkers, R., 2013. Potato Pedigree Database. Wageningen University and Research Centre, pp. 1–2. <https://www.wur.nl/en/show/Potato-Pedigree-Database-Updated.htm>.
- International Barley Genome Sequencing Consortium, 2012. A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491, 711–716.
- International Rice Genome Sequencing Project, 2005. The Map-based sequence of the rice genome. *Nature* 436, 793–800.
- Jaillon, O., Aury, J.-M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., Choisne, N., Aubourg, S., Vitulo, N., Jubin, C., et al., 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449, 463–467.
- JRTA, 2012. Encyclopedia of potato. In: *Japan Root and Tuber Crops Development Association Inc. Foundation Encyclopedia of Potato*, (Japan Root and Tuber Crops Development Association (JRTA)), pp. 167–168.
- Jupe, F., Witek, K., Verweij, W., Śliwka, J., Pritchard, L., Etherington, G.J., Maclean, D., Cock, P.J., Leggett, R.M., Bryan, G.J., et al., 2013. Resistance gene enrichment sequencing (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. *Plant J.* 76, 530–544.
- Kang, Y.J., Kim, K.H., Shim, S., Yoon, M.Y., Sun, S., Kim, M.Y., Van, K., Lee, S.-H., 2012. Genome-wide mapping of NBS-LRR genes and their association with disease resistance in soybean. *BMC Plant Biol.* 12, 139.
- Kozukue, N., Kozukue, E., Mizuno, S., 1987. Glycoalkaloids in potato plants and tubers. *Hortscience* 22, 294–296.
- Kroes, R., Renwick, A., Cheeseman, M., Kleiner, J., Mangelsdorf, I., Piersma, A., Schilter, B., Schlatter, J., van Schothorst, F., Vos, J., et al., 2004. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food Chem. Toxicol.* 42, 65–83.
- Lanfermeijer, F.C., Dijkhuis, J., Sturre, M.J.G., de Haan, P., Hille, J., 2003. Cloning and characterization of the durable tomato mosaic virus resistance gene *Tm-2<sup>2</sup>* from *Lycopersicon esculentum*. *Plant Mol. Biol.* 52, 1037–1049.
- Maga, J.A., 1994. Glycoalkaloids in solanaceae. *Food Rev. Int.* 10, 385–418.
- Malcolmson, J.F., Black, W., 1966. New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica* 15, 199–203.
- Marone, D., Russo, M.A., Laidò, G., De Leonardi, A.M., Mastrangelo, A.M., 2013. Plant Nucleotide Binding Site–Leucine-Rich Repeat (NBS-LRR) genes: active guardians in host defense responses. *Int. J. Mol. Sci.* 14, 7302–7326.
- McHale, L., Tan, X., Koehl, P., Micheltore, R.W., 2006. Plant NBS-LRR Proteins: adaptable guards. *Genome Biol.* 7, 1–11.
- Moffett, P., Farnham, G., Peart, J., Baulcombe, D.C., 2002. Interaction between domains of a plant NBS-LRR protein in disease resistance-related cell death. *EMBO J.* 21, 4511–4519.
- Monosi, B., Wissner, R.J., Pennill, L., Hulbert, S.H., 2004. Full-genome analysis of resistance gene homologues in rice. *Theoret. Appl. Gen.* 109, 1434–1447.
- Morel, J., Dangl, J.L., 1997. The hypersensitive response and the induction of cell death in plants. *Cell Death Differ.* 4, 671–683.
- Mucyn, T.S., Clemente, A., Andriotis, V.M.E., Balmuth, A.L., Oldroyd, G.E.D., Staskiewicz, B.J., Rathjen, J.P., 2006. The tomato NBARC-LRR protein prf interacts with Pto Kinase *in vivo* to regulate specific plant immunity. *Plant Cell* 18, 2792–2806.
- Mueller, L.A., Tanskey, S.D., Giovannoni, J.J., van Eck, J., Stack, S., Choi, D., Kim, B.D., Chen, M., Cheng, Z., Li, C., et al., 2005. The tomato sequencing project, the first cornerstone of the international solanaceae project (SOL). *Comp. Funct. Genom.* 6, 153–158.
- NDSU, 2017. North Dakota State University. Dakota Trailblazer. North Dakota State University Plant Sciences 6. <https://www.ag.ndsu.edu/plantsciences/research/releases/potato/dakota-trailblazer>.
- Nimchuk, Z., Eulgem, T., Holt III, B.F., Dangl, J.L., 2003. Recognition and response in the plant immune system. *Annu. Rev. Genet.* 37, 579–609.
- Novy, R.G., Love, S.L., Corsini, D.L., Pavek, J.J., Whitworth, J.L., Mosley, A.R., James, S.R., Hane, D.C., Shock, C.C., Rykboost, K.A., et al., 2006. Defender: a high-yielding, processing potato cultivar with foliar and tuber resistance to late blight. *Am. J. Potato Res.* 83, 9–19.
- Novy, R.G., Whitworth, J.L., Stark, J.C., Charlton, B.A., Yilma, S., Knowles, N.R., Pavek, M.J., Brandt, T.L., Gupta, S., Olsen, N., et al., 2012. Palisade russet: a late blight resistant potato cultivar having a low incidence of sugar ends and high specific gravity. *Am. J. Potato Res.* 89, 89–101.
- Novy, R.G., Whitworth, J.L., Stark, J.C., Schneider, B.L., Knowles, N.R., Pavek, M.J., Knowles, L.O., Charlton, B.A., Sathuvalli, V., Yilma, S., et al., 2017. Payette russet: a dual-purpose potato cultivar with cold-sweetening resistance, low acrylamide formation, and resistance to late blight and potato virus Y. *Am. J. Potato Res.* 94, 38–53.
- NPC, 2016. 2016 Potato Statistical Yearbook, vol. 1. National Potato Council, pp. 1–81.
- OECD, 1993. Organisation for Economic Co-operation and Development. Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles. <https://www.oecd.org/science/biotrack/41036698.pdf>.
- PAA, 2017. Kennebec (*Solanum tuberosum*), vol. 6. The Potato Association of America, pp. 1–3. <http://potatoassociation.org/industry/varieties/white-varieties/kennebec-Solanum-tuberosum>.
- Park, T., Gros, J., Sikkema, A., Vleeshouwers, V.G.A.A., Muskens, M., Allefs, S., Jacobsen, E., Visser, R.G.F., Van Der Vossen, E.A.G., 2005. The late blight resistance locus *Rpi-blb3* from *Solanum bulbocastanum* belongs to a major late blight R gene cluster on chromosome 4 of potato. *Mol. Plant Microbe Interact.* 18, 722–729.
- Pel, M.A., 2010. Mapping, Isolation and Characterization of Genes Responsible for Late Blight Resistance in Potato. Wageningen University.
- Pel, M.A., Foster, S.J., Park, T.-H., Rietman, H., van Arkel, G., Jones, J.D.G., Van Eck, H.J., Jacobsen, E., Visser, R.G.F., Van der Vossen, E.A.G., 2009. Mapping and cloning of late blight resistance genes from *Solanum venturii* using an interspecific candidate gene approach. *Mol. Plant Microbe Interact.* 22, 601–615.
- Pence, M., Spence, R., Rood, T., Habig, J., Collinge, S., 2016. United States Department of Agriculture Animal and Plant Health Inspection Service. Petition for Extension of Nonregulated Status for X17 Ranger Russet and Y9 Atlantic Potatoes with Late Blight Resistance, Low Acrylamide Potential, Lowered Reducing Sugars. USDA APHIS. [https://www.aphis.usda.gov/brs/aphisdocs/16\\_06401p.pdf](https://www.aphis.usda.gov/brs/aphisdocs/16_06401p.pdf).
- Potato Genome Sequencing Consortium, 2011. Genome sequence and analysis of the tuber crop potato. *Nature* 475, 189–195.
- Rietman, H., Bijsterbosch, G., Cano, L.M., Lee, H.-R., Vossen, J.H., Jacobsen, E., Visser, R.G.F., Kamoun, S., Vleeshouwers, V.G.A.A., 2012. Qualitative and quantitative late blight resistance in the potato cultivar sarp mira is determined by the perception of five distinct RXLR effectors. *Mol. Plant Microbe Interact.* 25, 910–919.

- Schmutz, J., Cannon, S.B., Schlueter, J., Ma, J., Mitros, T., Nelson, W., Hyten, D.L., Song, Q., Thelen, J.J., Cheng, J., et al., 2010. Genome sequence of the palaeopolyploid soybean. *Nature* 463, 178–183.
- Schnable, P.S., Ware, D., Fulton, R.S., Stein, J.C., Pasternak, S., Liang, C., Zhang, J., Fulton, L., Graves, T.A., Minx, P., et al., 2009. The B73 maize genome: complexity, diversity, and dynamics. *Science* 326, 1112–1115.
- Śliwka, J., Świątek, M., Tomczyńska, I., Stefańczyk, E., Chmielarz, M., Zimnoch-Guzowska, E., 2013. Influence of genetic background and plant age on expression of the potato late blight resistance gene *Rpi-phu1* during incompatible interactions with *Phytophthora infestans*. *Plant Pathol.* 62, 1072–1080.
- Song, W., Wang, B., Li, X., Wei, J., Chen, L., Zhang, D., Zhang, W., Li, R., 2015. Identification of immune related LRR-containing genes in maize (*Zea mays* L.) by genome-wide sequence analysis. *Internat. J. Gen.* 2015, 1–11.
- Spoel, S.H., Dong, X., 2012. How do plants achieve immunity? defence without specialized immune cells. *Nat. Rev. Immunol.* 12, 89–100.
- Stefanczyk, E., Sobkowiak, S., Brylinska, M., Sliwka, J., 2017. Expression of the potato late blight resistance gene *Rpi-phu1* and *Phytophthora infestans* effectors in the compatible and incompatible interactions in Potato. *Phytopathology* 107, 740–748.
- Stokes, T.L., Kunkel, B.N., Richards, E.J., 2002. Epigenetic variation in arabidopsis disease resistance. *Genes Dev.* 16, 171–182.
- Tan, M.Y.A., Hutten, R.C.B., Celis, C., Park, T.-H., Niks, R.E., Visser, R.G.F., van Eck, H.J., 2008. The *Rpi-mcd1* Locus from *Solanum microdontum* involved in resistance to *Phytophthora infestans*, causing a delay in infection, maps on potato chromosome 4 in a cluster of NBS-LRR genes. *Mol. Plant Microbe Interact.* 21, 909–918.
- Tan, X., Meyers, B.C., Kozik, A., West, M.A.L., Morgante, M., St Clair, D.a, Bent, A.F., Micheltore, R.W., 2007. Global expression analysis of nucleotide binding site-leucine rich repeat-encoding and related genes in *Arabidopsis*. *BMC Plant Biol.* 7, 56.
- The James Hutton Institute, 2017. Disease and Resistance in Potato. Disease-Resistance. pp. 1–5. <http://www.hutton.ac.uk/research/groups/cell-and-Molecular-Sciences/potato-Genetics/disease-Resistance>.
- Tomato Genome Consortium, 2012. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* 485, 635–641.
- Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., Fontana, P., Bhatnagar, S.K., Troggio, M., Pruss, D., et al., 2010. The genome of the domesticated apple (*Malus × domestica* Borkh.). *Nat. Genet.* 42, 833–839.
- VIB, 2017. A Late Blight Resistant Potato for Europe. <http://www.vib.be/en/about-vib/plant-biotech-news/>.
- Vleeshouwers, V.G.A.A., Raffaele, S., Vossen, J.H., Champouret, N., Oliva, R., Segretin, M.E., Rietman, H., Cano, L.M., Lokossou, A., et al., 2011. Understanding and exploiting late blight resistance in the age of effectors. *Annu. Rev. Phytopathol.* 49, 507–531.
- Vossen, J.H., van Arkel, G., Bergervoet, M., Jo, K.-R., Jacobsen, E., Visser, R.G.F.F., Van Arkel, G., Bergervoet, M., Ryong, K., Jacobsen, E., et al., 2016. The *Solanum demissum* R8 late blight resistance gene is an Sw-5 homologue that has been deployed worldwide in late blight resistant varieties. *Theoret. Appl. Gen.* 129, 1785.
- Wei, C., Chen, J., Kuang, H., 2016. Dramatic number variation of *r* genes in solanaceae species accounted for by a few *R* gene subfamilies. *PLoS One* 11, 1–15.
- WHO, 1995. World Health Organization. Application of the Principles of Substantial Equivalence to the Safety Evaluation of Foods or Food Components from Plants Derived by Modern Biotechnology. WHO/FNU/FOs 95.1. [http://apps.who.int/iris/bitstream/10665/58909/1/WHO\\_FNU\\_FOS\\_95.1.pdf](http://apps.who.int/iris/bitstream/10665/58909/1/WHO_FNU_FOS_95.1.pdf).
- Xu, C., Jiao, C., Sun, H., Cai, X., Wang, X., Ge, C., Zheng, Y., Liu, W., Sun, X., Xu, Y., et al., 2017. Draft genome of spinach and transcriptome diversity of 120 *Spinacia* accessions. *Nat. Commun.* 8, 15275.

1    **SUPPLEMENTAL TABLES**

2

3    **Supplemental Table 1S. Detection of R-Proteins in Plants: A Literature Review**

| R-protein | Class                    | Organism                                                  | Endogenous Protein Detected | Recombinant Protein Detected | Recombinant Expression                    | Reference                                  |
|-----------|--------------------------|-----------------------------------------------------------|-----------------------------|------------------------------|-------------------------------------------|--------------------------------------------|
| ADR1      | CC <sub>R</sub> -NBS-LRR | <i>Arabidopsis thaliana</i> ;<br><i>Solanum tuberosum</i> | No                          | Yes                          | HA-CC, strong promoter                    | Collier et al., 2011                       |
| ADR1-L1   | CC <sub>R</sub> -NBS-LRR | <i>Arabidopsis thaliana</i>                               | No                          | Yes                          | HA-CC, strong promoter                    | Collier et al., 2011                       |
| ADR1-L2   | CC <sub>R</sub> -NBS-LRR | <i>Arabidopsis thaliana</i>                               | No                          | Yes                          | HA-FL and CC, strong and native promoters | Collier et al., 2011; Roberts et al., 2013 |
| Bs2       | CC-NBS-LRR               | <i>Capsicum annuum</i>                                    | No                          | Yes                          | HA-CC, strong promoter                    | Collier et al., 2011                       |
| I-2       | CC-NBS-LRR               | <i>Solanum lycopersicum</i>                               | No                          | Yes                          | GST-NBS from inclusion bodies             | Tameling et al., 2002                      |
| L5:L6     | TIR-NBS-LRR              | <i>Linum usitatissimum</i>                                | No                          | Yes                          | HA-L5:L6 chimeras                         | Ravensdale et al., 2012                    |
| L6, L7    | TIR-NBS-LRR              | <i>Linum usitatissimum</i>                                | No                          | Yes                          | GAL4-AD:FL-L6, -L7 for Y2H                | Bernoux et al., 2011                       |
| M         | TIR-NBS-LRR              | <i>Linum usitatissimum</i>                                | No                          | Yes                          | His-FL -21 aa at N-term, inclusion body   | Schmidt et al., 2007                       |
| Mi-1      | CC-NBS-LRR               | <i>Solanum lycopersicum</i>                               | No                          | Yes                          | GST-NBS domain, inclusion body            | Tameling et al., 2002                      |
| N         | TIR-NBS-LRR              | <i>Nicotiana tabacum</i>                                  | No                          | Yes                          | HA-FL and TIR, strong promoter            | Mestre and Baulcombe, 2006                 |
| NRG1      | CC <sub>R</sub> -NBS-LRR | <i>Nicotiana benthamiana</i>                              | No                          | Yes                          | HA-FL and CC, strong promoter             | Peart et al., 2005                         |
| Prf       | SD*-CC-NBS-LRR           | <i>Solanum lycopersicum</i>                               | Yes                         | Yes                          | HA- and c-Myc-tagged, strong promoter     | Mucyn et al., 2006                         |
| Pi54of    | CC-NBS-LRR               | <i>Oryza officinalis</i>                                  | No                          | Yes                          | S-tagged                                  | Devanna et al., 2014                       |
| Pi-ta     | NBS-LRR                  | <i>Oryza sativa</i>                                       | No                          | Yes                          | S-tagged                                  | Jia et al., 2000                           |

|                   |                          |                             |    |     |                            |                             |
|-------------------|--------------------------|-----------------------------|----|-----|----------------------------|-----------------------------|
| RB                | CC-NBS-LRR               | <i>Solanum tuberosum</i>    | No | Yes | HA-CC, strong promoter     | Collier et al., 2011        |
| R3a               | CC-NBS-LRR               | <i>Solanum tuberosum</i>    | No | Yes | YFP- and c-Myc-tagged      | Engelhardt et al., 2012     |
| RGA4/5            | CC-NBS-LRR               | <i>Oryza sativa</i>         | No | Yes | HA-tagged                  | Cesari et al., 2013         |
| RPM1              | CC-NBS-LRR               | <i>Arabidopsis thaliana</i> | No | Yes | c-Myc-tagged               | Boyce et al., 1998          |
| RPP1              | TIR-NBS-LRR              | <i>Arabidopsis thaliana</i> | No | Yes | HA-tagged                  | Krasileva et al., 2010      |
| RPS2              | NBS-LRR                  | <i>Arabidopsis thaliana</i> | No | Yes | HA-tagged, native promoter | Axtell and Staskawicz, 2003 |
| RPW8              | CC <sub>R</sub> -NBS-LRR | <i>Arabidopsis thaliana</i> | No | Yes | HA-tagged                  | Wang et al., 2007           |
| Rx                | CC-NBS-LRR               | <i>Solanum tuberosum</i>    | No | Yes | HA-tagged                  | Bendahmane et al., 2002     |
| Tm-2 <sup>2</sup> | CC-NBS-LRR               | <i>Solanum lycopersicum</i> | No | Yes | Flag- and HA-LRR           | Du et al., 2013             |

\*SD = Solanaceous domain; antibodies detecting Prf target the SD, a domain not found in VNT1

## References for Table 1S.

- Axtell, M.J., and Staskawicz, B.J. (2003). Initiation of RPS2-Specified Disease Resistance in *Arabidopsis* Is Coupled to the AvrRpt2-Directed Elimination of RIN4. *Cell* 112, 369–377.
- Bendahmane, A., Farnham, G., Moffett, P., and Baulcombe, D.C. (2002). Constitutive Gain-of-Function Mutants in a Nucleotide Binding Site-Leucine Rich Repeat Protein Encoded at the *Rx* Locus of Potato. *The Plant Journal* 32, 195–204.
- Bernoux, M., Ve, T., Williams, S., Warren, C., Hatters, D., Valkov, E., Zhang, X., Ellis, J.G., Kobe, B., and Dodds, P.N. (2011). Structural and Functional Analysis of a Plant Resistance Protein TIR Domain Reveals Interfaces for Self-Association, Signaling, and Autoregulation. *Cell Host & Microbe* 9, 200–211.
- Boyes, D.C., Nam, J., and Dangl, J.L. (1998). The *Arabidopsis thaliana* RPM1 Disease Resistance Gene Product Is a Peripheral Plasma Membrane Protein That Is Degraded Coincident with the Hypersensitive Response. *Proceedings of the National Academy of Sciences* 95, 15849–15854.
- Cesari, S., Thilliez, G., Ribot, C., Chalvon, V., Michel, C., Jauneau, A., Rivas, S., Alaux, L., Kanzaki, H., Okuyama, Y., et al. (2013). The Rice Resistance Protein Pair RGA4/RGA5 Recognizes the *Magnaporthe oryzae* Effectors AVR-Pia and AVR1-CO39 by Direct Binding. *The Plant Cell* 25, 1463–1481.
- Collier, S.M., Hamel, L.P., and Moffett, P. (2011). Cell Death Mediated by the N-Terminal Domains of a Unique and Highly Conserved Class of NB-LRR Protein. *Molecular Plant-Microbe Interactions* 24, 918–931.
- Devanna, N.B., Vijayan, J., and Sharma, T.R. (2014). The Blast Resistance Gene *Pi54of* Cloned from *Oryza officinalis* Interacts with *Avr-Pi54* through Its Novel Non-LRR Domains. *PLoS One* 9, e104840.
- Du, Y., Zhao, J., Chen, T., Liu, Q., Zhang, H., Wang, Y., Hong, Y., Xiao, F., Zhang, L., Shen, Q., et al. (2013). Type I J-Domain NbMIP1 Proteins Are Required for Both *Tobacco mosaic virus* Infection and Plant Innate Immunity. *PLoS Pathogens* 9, e1003659.
- Engelhardt, S., Boevink, P.C., Armstrong, M.R., Ramos, M.B., Hein, I., and Birch, P.R.J. (2012). Relocalization of Late Blight Resistance Protein R3a to Endosomal Compartments Is Associated with Effector Recognition and Required for the Immune Response. *The Plant Cell* 24, 5142–5158.
- Jia, Y., McAdams, S.A., Bryan, G.T., Hershey, H.P., and Valent, B. (2000). Direct Interaction of Resistance Gene and Avirulence Gene Products Confers Rice Blast Resistance. *The EMBO Journal* 19, 4004–4014.
- Krasileva, K.V., Dahlbeck, D., and Staskawicz, B.J. (2010). Activation of an *Arabidopsis* Resistance Protein Is Specified by the in Planta Association of Its Leucine-Rich Repeat Domain with the Cognate Oomycete Effector. *The Plant Cell* 22, 2444–2458.
- Mestre, P., and Baulcombe, D.C. (2006). Elicitor-Mediated Oligomerization of the Tobacco N Disease Resistance Protein. *The Plant Cell* 18, 491–501.
- Mucyn, T.S., Clemente, A., Andriotis, V.M.E., Balmuth, A.L., Oldroyd, G.E.D., Staskawicz, B.J., and Rathjen, J.P. (2006). The Tomato NBARC-LRR Protein Prf Interacts with Pto Kinase *in Vivo* to Regulate

Specific Plant Immunity. *The Plant Cell* **18**, 2792–2806.

Peart, J.R., Mestre, P., Lu, R., Malcuit, I., and Baulcombe, D.C. (2005). NRG1, a CC-NB-LRR Protein, Together with N, a TIR-NB-LRR Protein, Mediates Resistance against Tobacco Mosaic Virus. *Current Biology* **15**, 968–973.

Ravensdale, M., Bernoux, M., Ve, T., Kobe, B., Thrall, P.H., Ellis, J.G., and Dodds, P.N. (2012). Intramolecular Interaction Influences Binding of the Flax L5 and L6 Resistance Proteins to Their AvrL567 Ligands. *PLoS Pathogens* **8**, e1003004.

Roberts, M., Tang, S., Stallmann, A., Dangl, J.L., and Bonardi, V. (2013). Genetic Requirements for Signaling from an Autoactive Plant NB-LRR Intracellular Innate Immune Receptor. *PLoS Genetics* **9**, e1003465.

Schmidt, S.A., Williams, S.J., Wang, C.-I.A., Sornaraj, P., James, B., Kobe, B., Dodds, P.N., Ellis, J.G., and Anderson, P.A. (2007). Purification of the M Flax-Rust Resistance Protein Expressed in *Pichia pastoris*. *The Plant Journal* **50**, 1107–1117.

Tameling, W.I.L., Elzinga, S.D.J., Darmin, P.S., Vossen, J.H., Takken, F.L.W., Haring, M.A., and Cornelissen, B.J.C. (2002). The Tomato R Gene Products I-2 and MI-1 Are Functional ATP Binding Proteins with ATPase Activity. *The Plant Cell* **14**, 2929–2939.

Wang, W., Devoto, A., Turner, J.G., and Xiao, S. (2007). Expression of the Membrane-Associated Resistance Protein RPW8 Enhances Basal Defense against Biotrophic Pathogens. *Molecular Plant-Microbe Interactions* **20**, 966–976.

77 **Supplemental Table 2S. Per Capita Acute and Chronic Potato Consumption from DEEM-FCID**

| Sub-population                     | 95 <sup>th</sup> percentile<br>Acute Residue<br>Consumption<br>(1ppm residue)<br>(mg/kg bw/d) <sup>1</sup> | 95 <sup>th</sup> Percentile<br>Acute Potato<br>Consumption<br>g/kg bw/d <sup>2</sup> | Chronic Residue<br>Consumption<br>(1ppm residue)<br>(mg/kg bw/d) <sup>1</sup> | Chronic Potato<br>Consumption<br>g/kg bw/d <sup>2</sup> |
|------------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------|
| Total U.S. Population <sup>3</sup> | 0.003045                                                                                                   | 3.05                                                                                 | 0.000682                                                                      | 0.68                                                    |
| Hispanic                           | 0.003004                                                                                                   | 3.00                                                                                 | 0.000640                                                                      | 0.64                                                    |
| Non-Hispanic-White                 | 0.003029                                                                                                   | 3.03                                                                                 | 0.000690                                                                      | 0.69                                                    |
| Non-Hispanic-Black                 | 0.003049                                                                                                   | 3.05                                                                                 | 0.000696                                                                      | 0.70                                                    |
| Non-Hispanic-Other                 | 0.003249                                                                                                   | 3.25                                                                                 | 0.000670                                                                      | 0.67                                                    |
| Nursing Infants                    | 0.002081                                                                                                   | 2.08                                                                                 | 0.000270                                                                      | 0.27                                                    |
| Non-Nursing Infants                | 0.004801                                                                                                   | 4.80                                                                                 | 0.000901                                                                      | 0.90                                                    |
| Female 13+ Pregnant                | 0.002762                                                                                                   | 2.76                                                                                 | 0.000659                                                                      | 0.66                                                    |
| Children 1-6                       | 0.006525                                                                                                   | 6.53                                                                                 | 0.001497                                                                      | 1.50                                                    |
| Children 7-12                      | 0.003966                                                                                                   | 3.97                                                                                 | 0.000929                                                                      | 0.93                                                    |
| Male 13-19                         | 0.003084                                                                                                   | 3.08                                                                                 | 0.000689                                                                      | 0.69                                                    |
| Female 13-19/Non-Pregnant          | 0.003001                                                                                                   | 3.00                                                                                 | 0.000636                                                                      | 0.64                                                    |
| Male 20+                           | 0.002742                                                                                                   | 2.74                                                                                 | 0.000619                                                                      | 0.62                                                    |
| Female 20+/Non-Pregnant            | 0.002318                                                                                                   | 2.32                                                                                 | 0.000513                                                                      | 0.51                                                    |
| Seniors 55+                        | 0.002466                                                                                                   | 2.47                                                                                 | 0.000570                                                                      | 0.57                                                    |
| All Infants                        | 0.003438                                                                                                   | 3.44                                                                                 | 0.000703                                                                      | 0.70                                                    |
| Female 13-50                       | 0.002395                                                                                                   | 2.40                                                                                 | 0.000533                                                                      | 0.53                                                    |
| Children 1-2 <sup>4</sup>          | 0.008185                                                                                                   | 8.19                                                                                 | 0.001719                                                                      | 1.72                                                    |
| Children 3-5                       | 0.005658                                                                                                   | 5.66                                                                                 | 0.001407                                                                      | 1.41                                                    |
| Children 6-12                      | 0.004406                                                                                                   | 4.41                                                                                 | 0.000982                                                                      | 0.98                                                    |
| Youth 13-19                        | 0.003049                                                                                                   | 3.05                                                                                 | 0.000662                                                                      | 0.66                                                    |
| Adults 20-49                       | 0.002513                                                                                                   | 2.51                                                                                 | 0.000557                                                                      | 0.56                                                    |
| Adults 50-99                       | 0.002506                                                                                                   | 2.51                                                                                 | 0.000576                                                                      | 0.58                                                    |
| Female 13-49                       | 0.002404                                                                                                   | 2.40                                                                                 | 0.000533                                                                      | 0.53                                                    |

<sup>1</sup>Parameters for the analysis: the default residue (ppm) was set to 1, and adjustment factor #1 was set to 5 for dry (granules/flakes), dry (granules/flakes) in baby food, flour, and flour in baby food to account for the fact that potatoes are 80% moisture (OECD, 2002).

<sup>2</sup>To convert residue consumption into potato consumption (g/kg bw/d) data for exposure assessment: i) Residue correction factor (x 1,000,000) was used to convert residue consumption (e.g. 1ppm) into potato consumption (mg/kg bw/d), and ii) unit conversion factor (x 0.001) was used to convert mg into g/kg bw/d.

<sup>3</sup>Green-highlighted cells indicate acute (95<sup>th</sup> percentile) potato consumption for the Total U.S. Population.

<sup>4</sup>Yellow-highlighted cells indicate acute (95<sup>th</sup> percentile) potato consumption for the sub-population with the highest exposure to potato.

80     **DEEM-FCID DETAILS**

81     Potato consumption data was obtained from the Dietary Exposure Evaluation Model – Food Commodity  
82     Intake Database (DEEM-FCID) (EPA, 2014). Version 10 of DEEM 4.02 was used. The potato consumption  
83     data include chips, dry (granules/flakes), dry (granules/flakes) in baby food, flour, flour in baby food,  
84     tubers without peel, tubers without peel in baby food, tubers with peel, and tubers with peel in baby  
85     food. It was assumed that, after adjustments, the amount of potatoes and potato products consumed  
86     were roughly equivalent to the amount of fresh weight potatoes consumed. Green and yellow acute  
87     (95<sup>th</sup> percentile) consumption numbers were used to calculate the amount of VNT1 consumed by the  
88     Total U.S. Population and Children Ages 1-2, respectively, from Y9 tubers.

89 **Supplemental Table 3S. Livestock Consumption of VNT1 from Late Blight Protected Potatoes**

| Livestock Type  | Feed Intake (dry matter in kg/d) | Potato Diet Incorporation (as fed) | Potato Diet Incorporation (dry matter) | Animal Body Weight (kg) | Potato Consumption (g dw/kg bw/d) <sup>6</sup> | VNT1 in Potatoes (µg/g dw) <sup>7</sup> | VNT1 Consumed (mg/kg bw/d) <sup>8</sup> |
|-----------------|----------------------------------|------------------------------------|----------------------------------------|-------------------------|------------------------------------------------|-----------------------------------------|-----------------------------------------|
| Cattle, beef    | 9.1 <sup>1</sup>                 | 30% <sup>1</sup>                   | 6% <sup>3</sup>                        | 500 <sup>1</sup>        | 1.1                                            | 2.5                                     | 0.0028                                  |
| Sheep, adult    | 1.5 <sup>2</sup>                 | 10% <sup>2</sup>                   | 2% <sup>3</sup>                        | 85 <sup>2</sup>         | 0.35                                           | 2.5                                     | 0.00088                                 |
| Sheep, lamb     | 2.0 <sup>2</sup>                 | 10% <sup>2</sup>                   | 2% <sup>3</sup>                        | 40 <sup>2</sup>         | 1.0                                            | 2.5                                     | 0.0025                                  |
| Pigs, finishing | 3.1 <sup>4</sup>                 | Not applicable                     | 30% <sup>5</sup>                       | 100 <sup>4</sup>        | 9.3                                            | 2.5                                     | 0.023                                   |

<sup>1</sup>From p. 28 of OECD, 2013<sup>2</sup>From p. 34 of OECD, 2013<sup>3</sup>“As fed” is converted to “dry matter” by dividing by 5, since potatoes are 20% solids and 80% water (OECD, 2002).<sup>4</sup>From p. 38 of OECD, 2013<sup>5</sup>From van Lunen et al., 1989<sup>6</sup>Feed intake in dry matter x % diet incorporation as dry matter x (1000 g/kg) / animal body weight<sup>7</sup>500 ppb VNT1 in fresh weight tubers = 0.5 µg/g in fresh weight tubers = 2.5 µg/g in dry weight tubers (since tubers are 20% solids and 80% water, OECD, 2002)<sup>8</sup>VNT1 consumed (mg/kg bw/d) = potato consumption (g dw potato/kg bw/d) x (2.5 µg VNT1/g dw tuber) x (0.001 mg/µg)