

September 24, 2021

Bernadette Juarez  
APHIS Deputy Administrator  
Biotechnology Regulatory Services  
Via email to: [RSRrequests@usda.gov](mailto:RSRrequests@usda.gov)

### Request for a Regulatory Status Review for BG25 Potato

Dear Ms. Juarez,

Please find attached a request for Regulatory Status Review for the potato event SPS-ØBG25-7 (BG25), in accordance with Part 340.4 (a)(4)(i) through (iii). BG25 has late blight protection, *Potato virus Y* protection, lower reducing sugars, and reduced black spot.

The submission includes:

- 1 PDF of the cover letter (no CBI);
- 1 PDF Justification for Confidential Business Information: the insert nucleotide sequence in BG25 (no CBI);
- 1 PDF of the RSR (CBI copy);
- 1 PDF of the RSR (CBI deleted copy); and
- 1 ZIP file with the cited references in PDF format (no CBI).

Thank you for your review of this request. Please contact me for any questions related to this submission.

Sincerely,

A handwritten signature in blue ink that reads "JPB Burzaco".

Juan P. Burzaco  
Regulatory Manager  
J.R. Simplot Company  
5369 West Irving Street  
Boise, ID 83706  
Tel.: (208) 780-7044  
[juan.burzaco@simplot.com](mailto:juan.burzaco@simplot.com)



**RECEIVED**

By caeck USDA APHIS BRS Document Control Officer at 5:28 pm, Sep 27, 2021

CBI Deleted Copy



**Request for a Regulatory Status Review for BG25 Potatoes with Late Blight Protection, *Potato virus Y* Protection, Lower Reducing Sugars, and Reduced Black Spot**

Prepared by Juan P. Burzaco\*

J.R. Simplot Company

OECD unique identifier: SPS-ØBG25-7 (BG25)

September 24, 2021

**\*Corresponding Author:**

Juan P. Burzaco  
J.R. Simplot Company  
5369 West Irving Street  
Boise, ID 83706  
Tel.: (208) 780-7044  
juan.burzaco@simplot.com

## Release of Information

J.R. Simplot Company is submitting the information in this document for review by the USDA as part of the Regulatory Status Review process established under USDA's regulations (7 CFR §340.4) implementing its authority under the Plant Protection Act, 7 U.S.C. § 7701. *et seq.* Simplot does not waive any rights to prevent its release to any third party. Were USDA to receive a request under the Freedom of Information Act (FOIA), 5 U.S.C. section 522, seeking all or some of the information in this submission, Simplot expects that, in advance of the release of any document(s) or information, USDA will provide Simplot with a copy of the material proposed to be released and the opportunity to object to the release of any information based upon appropriate legal grounds, e.g., responsiveness, confidentiality and/or competitive concerns. Simplot expects that no information that has been identified by Simplot as CBI (confidential business information) in accordance with 7 CFR § 340.7 will be provided to any third party. Simplot does not authorize the release, publication, or other distribution of its CBI (including website posting) without prior notice and consent from Simplot.

**Table of Contents**

Table of Figures .....4

List of Tables .....5

1.0 Introduction.....6

2.0 Description of the Comparator Plant .....8

3.0 Genotype of BG25 .....8

    3.1 Information on All Inserted Genetic Material .....8

        3.1.1 Genetic Sequences.....8

        3.1.2 Regulatory Sequences in BG25 .....14

        3.1.3 Molecular Characterization of the Insert in BG25 .....14

4.0 New Traits in BG25 .....17

    4.1 Phenotype of the New Traits and Mechanisms of Action.....17

        4.1.1 AMR3 and BLB2: Mechanism of Action .....17

        4.1.2 StmALS: Mechanism of Action .....18

    4.2 Metabolism, Physiology, and Development .....19

    4.3 Additional Information.....19

5.0 Summary.....19

References.....20

Appendix 1. Sequence of the Insert in BG25 .....i

**Table of Figures**

Figure 1. Plasmid Map of pSIM4363 ..... 9  
Figure 2. Sequence Alignment between StALS and StmALS ..... 13  
Figure 3. Insert Structure in BG25 with Junctions ..... 15  
Figure 4. Acetolactate Synthase Enzymatic Reaction ..... 18

**List of Tables**

Table 1. BG25 Traits, Phenotype, MOA, and Previous APHIS Review in Potato..... 7  
Table 2. Taxonomic Classification of Russet Burbank..... 8  
Table 3. Inserted Components in the pSIM4363 T-DNA..... 10  
Table 4. Characterization of Promoters and Terminators in BG25..... 14

## 1.0 Introduction

Simplot requests that BRS conduct a Regulatory Status Review (RSR) for Simplot potato Gen3 event SPS-ØBG25-7, hereafter referred to as BG25. BG25 event builds on Simplot's Gen1 and Gen2 potatoes by adding improvements to Russet Burbank, which is the #1 potato variety in the U.S., the premier storage variety, and excellent for many uses, including fresh and processing.

Compared to Gen2 potatoes (X17 and Y9), BG25 has two additional late blight resistance genes for more durable protection, and *Potato virus Y* (PVY) protection. Both late blight and PVY present significant disease concerns that impact potato yield and quality. According to USDA (2020), traits that assist in the control of these diseases would be beneficial to American agriculture. Late blight is a serious potato disease managed through frequent sprays of preventive fungicides. The introduction of late blight protection into commercial potato varieties enables reduced fungicide applications, with concomitant reductions in both costs and release of fungicide to the environment. Combining three R-proteins that recognize different effectors proteins reduces the chance that the pathogen can overcome the late blight protection and could improve durability of the late blight protection trait. PVY protection addresses a long-standing issue in the potato industry: the production of certified virus-free potato seed. PVY protection benefits the potato seed producer and also protects the grower from yield loss due to PVY infection and potato tuber necrotic ringspot disease.

BG25 also includes traits for lower reducing sugars and reduced black spot, potato-trait-mechanism of action combinations the agency has previously reviewed. Reducing sugars, which increase during storage, lead to dark colors in fries and worsen processing quality of tubers. Black spot is a physiological phenomenon resulting from the handling of potato tubers during harvest, transport, storage, and processing. The discoloration of tubers is undesirable for both consumers and processors and results in food waste.

The traits in BG25 potatoes were introduced through *Agrobacterium*-mediated transformation with plasmid pSIM4363. A modified potato (*Solanum tuberosum*) acetolactate synthase (StmALS) was used as a selection marker for transformed events and this protein is present in BG25. As noted, some of the potato-trait-mechanism of action (MOA) combinations present in BG25 have been previously reviewed and deregulated by APHIS (Table 1).

**Table 1. BG25 Traits, Phenotype, MOA, and Previous APHIS Review in Potato**

APHIS Trait	APHIS Phenotype	APHIS Mechanism of Action	Previously Reviewed by APHIS?	Reference Number
Fungal resistance	Resistance to potato late blight	VNT1 (late blight resistance gene from <i>Solanum venturii</i> ) protein recognizes Avr-vnt1 effector protein secreted by <i>Phytophthora infestans</i> and signals a hypersensitive response that destroys infected tissue through programmed cell death, restricting growth and spread of the pathogen to other parts of the plant.	Yes	14-093-01p 16-064-01p 19-099-02p
Fungal resistance	Resistance to potato late blight	Suggestion <sup>1</sup> : BLB2 (late blight resistance gene from <i>Solanum bulbocastanum</i> ) protein recognizes Avr-blb2 effector protein secreted by <i>Phytophthora infestans</i> and signals a hypersensitive response that destroys infected plant tissue through programmed cell death, restricting growth and spread of the pathogen to other parts of the plant.	No	Not applicable
Fungal resistance	Resistance to potato late blight	Suggestion <sup>1</sup> : AMR3 (late blight resistance gene from <i>Solanum americanum</i> ) protein recognizes Avr-amr3 effector protein secreted by <i>Phytophthora infestans</i> and signals a hypersensitive response that destroys infected plant tissue through programmed cell death, restricting growth and spread of the pathogen to other parts of the plant.	No	Not applicable
Virus resistance	Resistance to <i>Potato virus Y</i> (PVY)	RNAi mediated silencing of PVY coat protein gene.	Yes	97-339-01p
Altered tuber quality	Reduced black spot	Tuber specific RNAi mediated silencing of <i>Ppo5</i> (polyphenol oxidase-5) gene.	Yes	13-022-01p 14-093-01p 15-140-01p 16-064-01p 19-099-02p
Altered tuber sugar profile	Reduced glucose and fructose content in tubers	Tuber-specific RNAi mediated silencing of <i>Vinv</i> (vacuolar invertase) gene decreases VINV protein expression, resulting in less hydrolysis of sucrose to glucose and fructose.	Yes	14-093-01p 16-064-01p 19-099-02p
Marker gene (herbicide resistance)	Resistance to ALS inhibitor herbicides	An insensitive form of ALS (Acetolactate synthase/Acetoxy acid synthase) with a decreased binding affinity to ALS inhibitor herbicides.	No <sup>2</sup>	Not applicable

<sup>1</sup>USDA has not assigned a mechanism of action yet; this is suggested language.

<sup>2</sup>USDA has not reviewed this mechanism of action in potato; however, a modified ALS protein used as a selectable marker for transformation has been reviewed in soybean (06-354-01p). In addition, modified ALS proteins used as herbicide resistance traits have been reviewed in cotton, flax, soybean, corn, and canola.



To support this RSR request for BG25, the following information is provided in accordance with the requirements listed in Part 340.4 (a)(4)(i) through (iii):

- (i) A description of the comparator plant(s), to include genus, species, and any relevant subspecies information (Section 2.0);
- (ii) The genotype of the modified plant, including a detailed description of the differences in genotype between the modified and unmodified plant (Section 3.0); and
- (iii) A detailed description of the new trait(s) of the modified plant (Section 4.0).

This information supports that there is no plausible pathway to increased plant pest risk of BG25 compared to potatoes already on the market.

## 2.0 Description of the Comparator Plant

The taxonomic classification for the comparator plant, Russet Burbank, is provided in Table 2. Russet Burbank is the leading variety in acreage both in the United States and Canada. In 2020, Russet Burbank comprised 17.8% of seed potato acreage in the United States (Colorado State University, 2020) and 22.1% of seed potato hectares in Canada (CFIA, 2020). Russet Burbank is used as both a fresh and a processing potato and is known as the premier long-storage variety.

**Table 2. Taxonomic Classification of Russet Burbank**

Taxonomic Rank	Classification <sup>1</sup>
Family	Solanaceae
Genus	<i>Solanum</i>
Section	<i>petota</i>
Subsection	<i>potatoe</i>
Series	<i>tuberosa</i>
Species	<i>Solanum tuberosum</i>
Subspecies	<i>tuberosum</i>
Variety	Russet Burbank

<sup>1</sup>Based on OECD, 1997

## 3.0 Genotype of BG25

### 3.1 Information on All Inserted Genetic Material

#### 3.1.1 Genetic Sequences

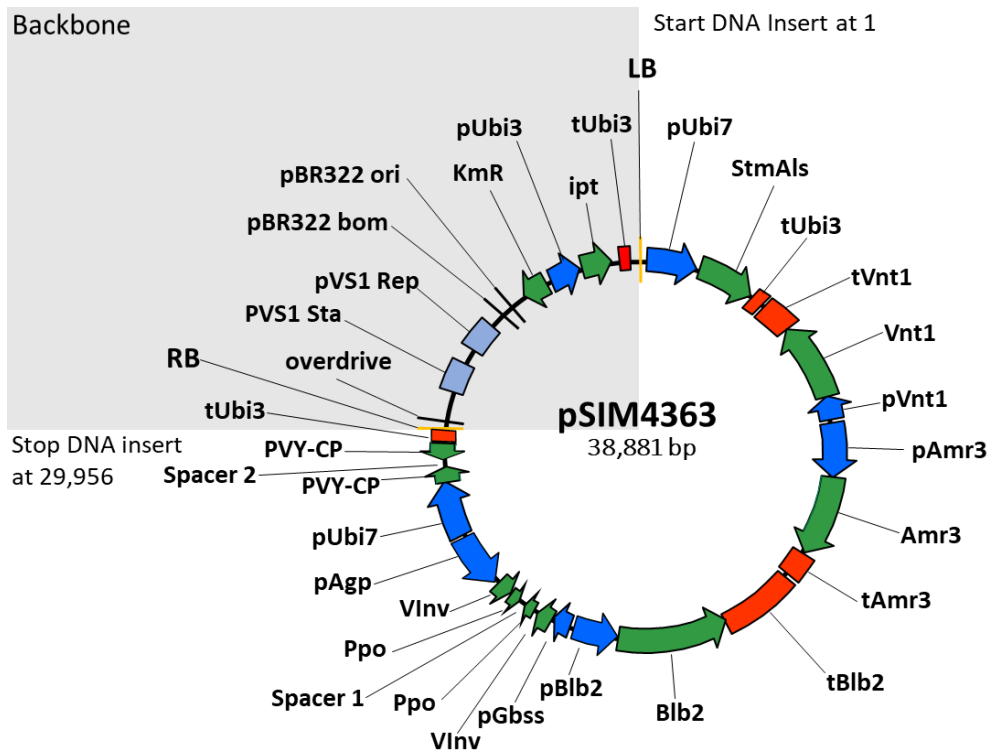
BG25 was generated by transforming Russet Burbank with the insert from pSIM4363 using *Agrobacterium* (Figure 1). BG25 was determined to be free of vector backbone DNA using qPCR. The name of inserted Component, construct component donor, accession number, nucleotide position, size

(bp), and function of the T-DNA elements in pSIM4363 are presented in Table 3. The nucleotide sequence for the inserted DNA in BG25 is confidential and provided in Appendix 1.

The T-DNA of pSIM4363 is 29,956 bp and consists of:

- Three R-genes (*Rpi-vnt1*, *Rpi-amr3*, *Rpi-blb2*) from wild Solanum species for foliar and tuber protection against late blight;
- A PVY sequence that uses RNAi to target the coat protein region of the PVY genome for PVY protection;
- Sequences from Solanum species for down regulation of potato polyphenol oxidase (*Ppo*) and potato vacuolar invertase (*VInv*) transcripts using RNAi; and
- A modified potato acetolactate synthase gene (*StmAIs*) as a selectable marker for transformation.

One of the inserted sequences, the *StmAIs* gene, has been modified from its native form. An alignment of the modified sequence with the unmodified sequence is provided in Figure 2.



**Figure 1. Plasmid Map of pSIM4363**

The T-DNA region (white) is from 1 bp to 29,956 bp. The T-DNA is from potato species except for the PVY-CP sequence (1,044 bp) and left and right borders. The backbone region (gray) starts at 29,957 bp and ends at 38,881 bp. The backbone consists of bacterial and potato DNA.

**Table 3. Inserted Components in the pSIM4363 T-DNA**

Name of Inserted Component	Construct Component Donor	Accession Number	Nucleotide Position	Size (bp)	Function
1. Left border (LB) region					
A. LB site	Synthetic DNA	AY566555	1-25	25	Secondary cleavage site releases ssDNA inserts from pSIM4363
B. LB buffer	<i>S. tuberosum</i> var. Ranger Russet.	AY566555	26-187	162	Buffer for truncations during insertion
2. Intervening sequence	<i>S. tuberosum</i>	AF393847	188-192	5	Sequence used for DNA cloning
3. Polyubiquitin promoter (pUbi7)	<i>S. tuberosum</i> var. Ranger Russet	U26831	193-1,928	1,736	Drives expression of the <i>StmAls</i> gene
4. Intervening sequence	Synthetic DNA	-	1,929-1,934	6	Sequence used for DNA cloning
5. Modified <i>Als</i> gene ( <i>StmAls</i> )	<i>S. tuberosum</i>	XM_006361678	1,935-3,914	1,980	Selection marker; modified potato ALS ( <i>StmALS</i> )
6. Intervening sequence	Synthetic DNA	-	3,915-3,920	6	Sequence used for DNA cloning
7. Ubiquitin-3 terminator (tUbi3)	<i>S. tuberosum</i>	GP755544	3,921-4,275	355	Terminates transcription of <i>StmAls</i> gene
8. Intervening sequence	Synthetic DNA	-	4,276-4,285	10	Sequence used for DNA cloning
9. <i>Rpi-vnt1</i> native terminator (tVnt1)	<i>S. venturii</i>	FJ423044	4,286-5,210	925	Terminates transcription of <i>Rpi-vnt1</i>
10. <i>Rpi-vnt1</i> gene coding sequence ( <i>Vnt1</i> )	<i>S. venturii</i>	FJ423044	5,211-7,886	2,676	Expresses the VNT1 protein for foliar late blight protection
11. <i>Rpi-vnt1</i> native promoter (pVnt1)	<i>S. venturii</i>	FJ423044	7,887-8,595	709	Drives expression of the <i>Rpi-vnt1</i>
12. Intervening sequence	Synthetic DNA	-	8,596-8,601	6	Sequence used for DNA cloning
13. <i>Rpi-amr3</i> native promoter (pAmr3)	<i>S. americanum</i>	KT373889.1	8,602-10,519	1,918	Drives expression of the <i>Rpi-amr3</i> gene
14. <i>Rpi-amr3</i> gene coding sequence ( <i>Amr3</i> )	<i>S. americanum</i>	KT373889.1	10,520-13,183	2,664	Expresses the AMR3 protein for late blight protection
15. <i>Rpi-amr3</i> native terminator (tAmr3)	<i>S. americanum</i>	KT373889.1	13,184-13,953	770	Terminates transcription of <i>Rpi-amr3</i> gene
16. Intervening sequence	Synthetic DNA	-	13,954-13,965	12	Sequence used for DNA cloning
17. <i>Rpi-blb2</i> native terminator (tBlb2)	<i>S. bulbocastanum</i>	DQ122125	13,966-16,497	2,532	Terminates transcription of <i>Rpi-blb2</i> gene
18. <i>Rpi-blb2</i> gene coding sequence ( <i>Blb2</i> )	<i>S. bulbocastanum</i>	DQ122125	16,498-20,387	3,890	Expresses the BLB2 protein for late blight protection
19. <i>Rpi-blb2</i> native promoter (pBlb2)	<i>S. bulbocastanum</i>	DQ122125	20,388-21,932	1,545	Drives expression of the <i>Rpi-blb2</i> gene
20. Intervening sequence	Synthetic DNA	-	21,933-21,940	8	Sequence used for DNA cloning
21. Granule-bound starch synthase promoter (pGbss)	<i>S. tuberosum</i> var. Ranger Russet	HM363755	21,941-22,626	686	Drives expression of the VInv/Ppo inverted repeat

**Table 3. Inserted Components in the pSIM4363 T-DNA (continued)**

Name of Inserted Component	Construct Component Donor	Accession Number	Nucleotide Position	Size (bp)	Function
22. Intervening sequence	Synthetic DNA	-	22,627-22,633	7	Sequence used for DNA cloning
23. Vacuolar invertase gene fragment (sense orientation; <i>VInv</i> )	<i>S. tuberosum</i> var. Ranger Russet	DQ478950	22,634-23,137	504	Generates dsRNA to down regulate <i>VInv</i> transcripts
24. Intervening sequence	Synthetic DNA	-	23,138-23,143	6	Sequence used for DNA cloning
25. 3'-untranslated region of the polyphenol oxidase gene (antisense orientation; <i>Ppo</i> )	<i>S. verrucosum</i>	HM363754	23,144-23,287	144	Generates dsRNA to down regulate <i>Ppo</i> transcripts
26. Spacer 1	<i>S. tuberosum</i> var. Ranger Russet	HM363753	23,288-23,450	163	Sequence between the inverted repeats; forms loop in dsRNA transcript
27. 3'-untranslated region of the polyphenol oxidase gene (sense orientation; <i>Ppo</i> )	<i>S. verrucosum</i>	HM363754	23,451-23,594	144	Forms dsRNA to reduce expression of polyphenol oxidase to reduce black spot
28. Intervening sequence	Synthetic DNA	-	23,595-23,601	7	Sequence used for DNA cloning
29. Vacuolar invertase gene fragment (antisense orientation, <i>VInv</i> )	<i>S. tuberosum</i> var. Ranger Russet	DQ478950	23,602-24,099	498	Generates dsRNA to down regulate <i>VInv</i> transcripts
30. Intervening sequence	Synthetic DNA	-	24,100-24,105	6	Sequence used for DNA cloning
31. ADP glucose pyrophosphorylase promoter (pAgp)	<i>S. tuberosum</i> var. Ranger Russet	HM363752	24,106-26,365	2,260	Drives expression of <i>VInv</i> / <i>Ppo</i> inverted repeat
32. Intervening sequence	Synthetic DNA	-	26,366-26,377	12	Sequence used for DNA cloning
33. Polyubiquitin promoter (pUbi7)	<i>S. tuberosum</i> var. Ranger Russet	U26831	26,378-28,113	1,736	Drives expression of the PVY inverted repeat
34. Intervening sequence	Synthetic DNA	-	28,114-28,119	6	Sequence used for DNA cloning
35. <i>Potato virus Y</i> coat protein gene fragment (sense orientation, PVY-CP)	<i>Potato virus Y</i> strain N	AJ890342	28,120-28,641	522	Generates dsRNA that target PVY genome for degradation
36. Spacer 2	<i>S. tuberosum</i> var. Ranger Russet	HM363755	28,642-28,878	237	Gbss intron sequence forms loop in dsRNA transcript
37. <i>Potato virus Y</i> coat protein gene fragment (antisense orientation, PVY-CP)	<i>Potato virus Y</i> strain N	AJ890342	28,879-29,400	522	Generates dsRNA that target PVY genome for degradation
38. Intervening sequence	Synthetic DNA	-	29,401-29,406	6	Sequence used for DNA cloning
39. Ubiquitin-3 terminator (tUbi3)	<i>S. tuberosum</i>	GP755544	29,407-29,761	355	Terminator for PVY-CP dsRNA transcript

**Table 3. Inserted Components in the pSIM4363 T-DNA (continued)**

Name of Inserted Component	Construct Component Donor	Accession Number	Nucleotide Position	Size (bp)	Function
40. Intervening sequence	Synthetic DNA	-	29,762-29,770	9	Sequence used for DNA cloning
41. Right border (RB) region		-			
A. RB buffer	<i>S. tuberosum</i> var. Ranger Russet	AY566555	29,771-29,931	161	Supports primary cleavage at RB
B. RB site	Synthetic	AY566555	29,932-29,956	25	Primary cleavage site releases ssDNA insert from pSIM4363

Modified Inserted Sequence: StmALS

BG25 contains a modified version of the *S. tuberosum* acetolactate synthase gene (*StmAls*), which is used as a selection marker during transformation. The full-length StmALS is 659 amino acids, and StmALS differs from StALS by two amino acid substitutions: the tryptophan residue at 563 changed to leucine (W563L), and the serine residue at 642 changed to isoleucine (S642I). An alignment of the modified with the unmodified sequence is provided in Figure 2.

The mechanism of action of StmALS is explained in Section 4.1.2.

```

StALS      MAAAASPPSPCFSKTLPSSSSKSSSTILPRSTFFPHNHPQKASPLHLTHTHHRRGFVAVSNV 60
StmALS     MAAAASPPSPCFSKTLPSSSSKSSSTILPRSTFFPHNHPQKASPLHLTHTHHRRGFVAVSNV 60
*****

StALS      VISTTTHNDVSEPETFVSRFAPDEPRKGCVDLVEALEREGVTDVFAYPPGGASMEIHQALT 120
StmALS     VISTTTHNDVSEPETFVSRFAPDEPRKGCVDLVEALEREGVTDVFAYPPGGASMEIHQALT 120
*****

StALS      RSNIIRNVLPRHQQGGVFAAEGYARATGFPGVCIATSGPGATNLVSGLADALLDSIPIVA 180
StmALS     RSNIIRNVLPRHQQGGVFAAEGYARATGFPGVCIATSGPGATNLVSGLADALLDSIPIVA 180
*****

StALS      ITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVEDIPRVVREAFFLAKSGRPGPVL 240
StmALS     ITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVEDIPRVVREAFFLAKSGRPGPVL 240
*****

StALS      IDVPKDIQQQLVIPNWDQPMRLPGYMSRLPKLPNEMLLEQIIRLISESKKPVLYVGGGCL 300
StmALS     IDVPKDIQQQLVIPNWDQPMRLPGYMSRLPKLPNEMLLEQIIRLISESKKPVLYVGGGCL 300
*****

StALS      QSSEELRRFVELTGIPVASTLMGLGAFPTGDELSLQMLGMHGTVYANYAVDGSDDL LAFG 360
StmALS     QSSEELRRFVELTGIPVASTLMGLGAFPTGDELSLQMLGMHGTVYANYAVDGSDDL LAFG 360
*****

StALS      VRFDDRVTGKLEAFASRAKIVHIDIDS A EIGKNKQPHVSI CADIKLALQGLNSILEGKEG 420
StmALS     VRFDDRVTGKLEAFASRAKIVHIDIDS A EIGKNKQPHVSI CADIKLALQGLNSILEGKEG 420
*****

StALS      KLKLDFAWRQELTEQKVKYP LSFKTFGEAIP P QYAIQV LDEL TNGNAIISTGVGQHQM W 480
StmALS     KLKLDFAWRQELTEQKVKYP LSFKTFGEAIP P QYAIQV LDEL TNGNAIISTGVGQHQM W 480
*****

StALS      AAQYYKYKKPHQWLTS GGLGAMGFGLPAAIGAAVGRPGEIVVDIDGDGSFIMNVQELATI 540
StmALS     AAQYYKYKKPHQWLTS GGLGAMGFGLPAAIGAAVGRPGEIVVDIDGDGSFIMNVQELATI 540
*****

StALS      KVENLPVKIMLLNNQH LGMVVQ WEDRFYKANRAHTY LGDPANEEEEIFPNMLKF AEACGVP 600
StmALS     KVENLPVKIMLLNNQH LGMVVQ L EDRFYKANRAHTY LGDPANEEEEIFPNMLKF AEACGVP 600
*****

StALS      AARVSHRDDLR AAIQKMLDTPGPYLLDVIVPHQEHLVPMIP SGGAFKDVITEGDGRSY 659
StmALS     AARVSHRDDLR AAIQKMLDTPGPYLLDVIVPHQEHLVPMIP IGGAFKDVITEGDGRSY 659
*****

```

**Figure 2. Sequence Alignment between StALS and StmALS**

The native, full-length potato acetolactate synthase (StALS) protein sequence (Accession: ADI56521) was aligned to the modified StmALS sequence in BG25 using Clustal Omega (Sievers et al., 2011). The modified potato ALS (StmALS) has two amino acid substitutions (W563L, S642I; highlighted in red) and is 99.7% identical compared to the native potato ALS (StALS).

### 3.1.2 Regulatory Sequences in BG25

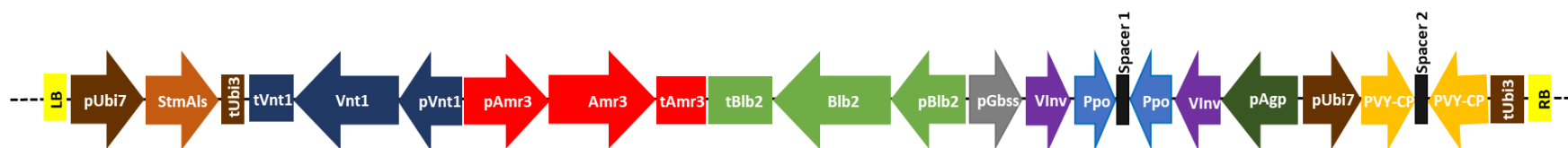
This section further characterizes the regulatory sequences in BG25, which consist of six promoters and four terminators (Table 4). The function and source for each sequence were presented in Table 3.

**Table 4. Characterization of Promoters and Terminators in BG25**

Regulatory Sequence	Associated Expressed Sequence	Characterization	Previously Reviewed in Deregulated Simplot Events	Reference line to Table 3
<b>Promoters</b>				
Potato polyubiquitin (pUbi7)	<i>StmAls</i> gene	Constitutive (Garbarino et al., 1995)	No	3
	PVY-CP inverted repeat			33
Native <i>Rpi-vnt1</i> (pVnt1)	<i>Rpi-vnt1</i> gene	Inducible by <i>P. infestans</i> (Karasov et al., 2017)	Yes	11
Native <i>Rpi-amr3</i> (pAmr3)	<i>Rpi-amr3</i> gene		No	13
Native <i>Rpi-blb2</i> (pBlb2)	<i>Rpi-blb2</i> gene		No	19
Potato granule-bound starch synthase (pGbss)	Vlnv/Ppo inverted repeat	Active in leaves and tubers (Nakata et al., 1994)	Yes	21
Potato ADP glucose pyrophosphorylase (pAgp)		Highly active in tubers and stolons, with lower expression in leaves (Nakata et al., 1994)	Yes	31
<b>Terminators</b>				
Ubiquitin-3 (tUbi3)	<i>StmAls</i> gene	Native potato terminator	No	7
	PVY-CP inverted repeat			39
<i>Rpi-vnt1</i> (tVnt1)	<i>Rpi-vnt1</i> gene	Native <i>Solanum</i> terminator	Yes	9
<i>Rpi-amr3</i> (tAmr3)	<i>Rpi-amr3</i> gene	Native <i>Solanum</i> terminator	No	15
<i>Rpi-blb2</i> (tBlb2)	<i>Rpi-blb2</i> gene	Native <i>Solanum</i> terminator	No	17

### 3.1.3 Molecular Characterization of the Insert in BG25

The structure of the pSIM4363 insert in BG25 is shown in Figure 3.



**Figure 3. Insert Structure in BG25 with Junctions**

The BG25 event contains a single, nearly full-length T-DNA from pSIM4363. The plant genomic sequence is indicated with a dashed line.



### Single Insert

A combination of Sanger sequencing and Illumina Next Generation Sequencing (NGS) and corroborating studies using droplet digital PCR (ddPCR) showed the presence of a single insert in BG25 following transformation of Russet Burbank with pSIM4363. The ddPCR measured the number of copies of eight unique junctions spanning the insert in the BG25 genome. Collectively, the data indicated that each unique junction was present in BG25 as a single copy at a single locus, which was shown to be on chromosome 12. No annotated genes were disrupted by the insert and the BG25 genome has 55 bp deleted at the insertion site. The sequence of the insert is confidential and is provided in Appendix 1. The insert is a nearly full-length T-DNA, with a 139 bp deletion on the 5' end and a 34 bp deletion on the 3' end. No backbone DNA was integrated into the BG25 genome.

The BG25 Illumina data were generated by sequencing mate pair DNA fragments sharing sequence identity to pSIM4363 after targeted capture. Junction-finding scripts using these sequences and the DM potato reference genome (Potato Genome Sequencing Consortium, 2011; Sharma et al., 2013), specifically PGSC *S. tuberosum* group Phureja clone DM1-3 pseudomolecules (v4.03), indicated a single insertion site, corroborating T-DNA copy number by ddPCR. Sanger sequencing was performed on PCR products across the junctions including at least 1 kb of flanking DNA near the left border and right border. These sequences were assembled with the plasmid T-DNA sequence to obtain the sequence for the insertion site.

To inspect and validate the proposed insert and flanking sequence, Illumina reads from mate pair libraries after targeted capture were aligned to the plasmid sequence as well as the tetraploid potato Russet Burbank draft genome (v1.0). The Russet Burbank draft genome (v1.0) was developed in collaboration with Corteva™ Agriscience (Johnston, IA), utilizing PacBio CLR sequencing reads (Sequel II) with an average length of 34 kb to develop a genome assembly from ~37x coverage of the tetraploid genome. The enrichment yielded thorough depth of coverage (average 99x) across the entire pSIM4363 insert, including the junctions and adjacent flanking genomic DNA. Combined sequencing data showed that the pSIM4363 insert contains a nearly full-length T-DNA, with deletions of 139 bp from the annotated left border element and 34 bp from the annotated right border element.

### Integration Site

The sequence of the genomic integration site for the insert in BG25 was determined using primers that hybridize to the genomic regions flanking the insert. PCR with these primers amplified the homologous, native loci in Russet Burbank. By comparing the Sanger-sequenced insertion site with the insert described above, the pSIM4363 insertion site in BG25 was identified. These data indicated that 55 bp of genomic DNA were deleted as a result of the pSIM4363 insertion. These data also showed that the flanking sequence has identity to the DM potato reference genome (v4.03) chromosome 12 and is not located in any annotated gene.

### Absence of Backbone

Illumina sequencing and PCR analysis were used to show the absence of plasmid backbone in BG25. Illumina mate pair sequencing libraries were captured to enrich for high coverage of sequences derived from pSIM4363, including the backbone. These results showed that no backbone from the plasmid was inserted in BG25. In addition, six PCR assays were designed across the plasmid backbone of pSIM4363. Using the potato gene adenine phosphoribosyl transferase (APRT) as a positive, endogenous control, no amplification was observed for the plasmid backbone using DNA from BG25.

## 4.0 New Traits in BG25

### 4.1 Phenotype of the New Traits and Mechanisms of Action

BG25 has late blight protection, PVY protection, lower reducing sugars, and reduced black spot. The potato-trait-MOA combinations of late blight protection (from VNT1), PVY protection, lower reducing sugars, and reduced black spot, have been previously reviewed and deregulated by APHIS (Table 1). These plant-trait-MOA can be found on the APHIS § 340.1(c) exemption list and therefore qualify for exemption.

A PVY-resistant potato event, assessed by APHIS under petition 97-339-01p, expressed a viral coat protein, but the mechanism was not understood at that time (Kaniewski and Thomas, 2004). A later review suggested the mechanism was likely to be RNA based and not a result of protein expression (Rosa et al., 2018). Simplot proposes the following language to describe the PVY protection MOA for BG25:

“Small interfering RNA target the coat protein region of the PVY genome and inhibit viral replication.”

The new trait-MOA combinations in BG25 include protection against late blight infection from two new R-genes: *Rpi-amr3* and *Rpi-blb2*. Although the *StmALS* is not a commercial herbicide tolerance trait in BG25, it is a new trait-MOA for potato. Therefore, the new plant-trait-MOA combinations in BG25 are:

- Late blight protection using AMR3 R-protein;
- Late blight protection using BLB2 R-protein; and
- Selectable marker using *StmALS*.

#### 4.1.1 AMR3 and BLB2: Mechanism of Action

Like VNT1, the AMR3 and BLB2 R-proteins are part of a plant defense mechanism called effector-triggered immunity. They recognize pathogen-secreted effector proteins and activate the plant hypersensitive response (HR). The plant HR is a form of programmed plant cell death that kills infected cells (apoptosis) and blocks spread of infection to the rest of the plant (Jones and Dangl, 2006). R-proteins do not have a pesticidal mechanism of action since they do not act on the invading pathogen (Panstruga et al., 2009).

The expressed AMR3 protein in BG25 recognizes the *Avr-amr3* effector produced by *P. infestans* (Witek et al., 2016). This recognition initiates the host HR resulting in late blight protection. More details about how the *Avr-amr3* effector functions have yet to be determined.

The expressed BLB2 protein in BG25 recognizes the *Avr-blb2* effector produced by *P. infestans* (Oh et al., 2009, 2014). This recognition initiates the host HR resulting in late blight protection. *Avr-blb2* is secreted by *P. infestans* and is localized at the cell membrane or in the haustoria at the pathogen-generated secretory interface (Bozkurt et al., 2011). In the plant cell, *Avr-blb2* binds calmodulin, a calcium-binding protein, suggesting it may function to disrupt  $Ca^{2+}$  signaling during pathogen infection (Naveed et al., 2019). In the absence of BLB2, *Avr-blb2* may block the host plant's ability to secrete immune proteases, which would dampen the plant's immune response (Bozkurt et al., 2011).

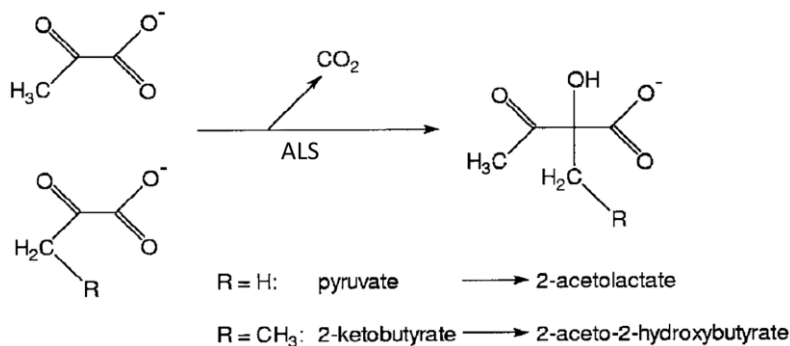
In summary, the AMR3 and BLB2 proteins have the same mechanism of action as VNT1, except that each of the three proteins recognizes a different pathogen-secreted effector protein. Combining three

R-proteins that recognize different effectors proteins reduces the chance that the pathogen can overcome the late blight protection and could improve durability of the late blight protection trait.

#### 4.1.2 StmALS: Mechanism of Action

Acetolactate synthase (ALS), also known as acetohydroxyacid synthase (AHAS), is an enzyme expressed in bacteria, fungi, algae, and all plant species, but not in animals. ALS catalyzes the first common step in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine in plants (McCourt and Duggleby, 2006). Humans do not have this pathway and must obtain these amino acids from their diet.

The native potato *StAls* gene, which includes a chloroplast transit sequence, encodes a 659 amino acid polypeptide that interacts with a second ALS polypeptide to form a homodimer. This homodimer is the active form of the enzyme. ALS converts two pyruvate molecules to 2-acetolactate (a precursor of leucine and valine), or pyruvate and 2-ketobutyrate to 2-aceto-2-hydroxy-butyrate (a precursor of isoleucine; Figure 4).



#### Figure 4. Acetolactate Synthase Enzymatic Reaction

Acetolactate synthase (ALS) converts two molecules of pyruvate to 2-acetolactate, or converts pyruvate and 2-ketobutyrate to 2-aceto-2-hydroxy-butyrate (modified from Duggleby and Pang, 2000).

ALS-inhibiting herbicides inhibit branched chain amino acid synthesis by binding to ALS and blocking substrate (i.e., pyruvate or 2-ketobutyrate) access to the active site (McCourt et al., 2006). By blocking the first step in branched chain amino acid synthesis, these ALS inhibitors cause a deficiency in amino acids necessary for growth and survival, and an accumulation of 2-ketobutyrate (Duggleby and Pang, 2000), which result in the death of the plant.

BG25 contains a modified version of the *S. tuberosum* acetolactate synthase gene (*StmAls*), which is used as a selection marker during transformation. The full-length *StmALS* is 659 amino acids and has a predicted molecular weight of approximately 72 kDa. The native potato ALS (*StALS*) is sensitive to ALS-inhibiting herbicides (e.g., sulfonylureas and imidazolinone) that bind to the protein and block its activity. This causes the plant to die from the lack of essential branched-chain amino acids. The *StmALS* differs from *StALS* by two amino acid substitutions: the tryptophan residue at 563 changed to leucine (W563L), and the serine residue at 642 changed to isoleucine (S642I). The two amino acid substitutions in *StmALS* (Figure 2) interfere with herbicide binding and enable the *StmALS* protein to be resistant to ALS-inhibiting herbicides. The substrates enter the enzyme active site even in the presence of the herbicide, ensuring the synthesis of branched chain amino acids.

StmALS has the same mechanism of action as GM-HRA present in soybean, a trait already deregulated by the APHIS. In addition, modified ALS proteins with the same mechanism of action have been deregulated in herbicide tolerant cotton, flax, soybean, corn, and canola (Table 1). Herbicide tolerance is not a commercial trait in BG25, and Simplot will not claim resistance to ALS-inhibiting herbicides.

#### **4.2 Metabolism, Physiology, and Development**

None of the new traits in BG25 have an impact on metabolism, physiology, or development of the plant.

#### **4.3 Additional Information**

An Experimental Use Permit is being reviewed by the Environmental Protection Agency (EPA) for plasmid pSIM4363 (8917-EUP-G), which was used to develop BG25. In addition, tolerance exemptions for AMR3 and BLB2 (OG8830), and StmALS (IN-11411) are being reviewed by the EPA. Simplot intends to consult with the Food and Drug Administration on the use of BG25 in food and feed.

#### **5.0 Summary**

BG25 is the first Gen3 potato variety from Simplot, introducing more durable late blight protection and PVY protection which will benefit American agriculture by reducing fungicide use, improving seed quality, and helping protect yield. Like previous Innate® events, BG25 also has lower reducing sugars, which contributes to better processing quality, and reduced black spot, which contributes to reduced food waste.

Several of the potato-trait-MOA combinations present in BG25 have been previously evaluated and deregulated by USDA (late blight protection (VNT1), Potato virus Y protection, lower reducing sugars, and reduced black spot; Table 1). The novel potato-trait-MOA combinations in BG25 include late blight protection using AMR3 and BLB2 R-proteins, and StmALS as a selectable marker. BG25 potato event presents no plausible pathway for the plant, or its sexually compatible relatives, to pose increased plant pest risk compared to potatoes already on the market.

## References

- Bozkurt, T.O., Schornack, S., Win, J., Shindo, T., Ilyas, M., Oliva, R., Cano, L.M., Jones, A.M.E., Huitema, E., Van Der Hoorn, R.A.L., Kamoun, S., 2011. *Phytophthora infestans* effector AVRblb2 prevents secretion of a plant immune protease at the haustorial interface. *Proc. Natl. Acad. Sci. U. S. A.* 108, 20832–20837. <https://doi.org/10.1073/pnas.1112708109>
- CFIA, 2020. 2020 Total Certified Potato Seed Hectares.
- Colorado State University, 2020. 2020 Total Certified Seed Accepted Acres - USA.
- Duggleby, R.G., Pang, S.S., 2000. Acetohydroxyacid Synthase. *J. Biochem. Mol. Biol.* 33, 1–36. <https://doi.org/10.1107/S0907444901011635>
- Garbarino, J.E., Oosumi, T., Belknap, W.R., 1995. Isolation of a polyubiquitin promoter and its expression in transgenic potato plants. *Plant Physiol.* 109, 1371–8.
- Jones, J.D.G., Dangl, J.L., 2006. The Plant Immune System. *Nature* 444, 323–9. <https://doi.org/10.1038/nature05286>
- Kaniewski, W.K., Thomas, P.E., 2004. The potato story. *AgBioForum* 7, 41–46.
- Karasov, T.L., Chae, E., Herman, J.J., Bergelson, J., 2017. Mechanisms to mitigate the trade-off between growth and defense. *Plant Cell* 29, 666–680. <https://doi.org/10.1105/tpc.16.00931>
- McCourt, J.A., Duggleby, R.G., 2006. Acetohydroxyacid synthase and its role in the biosynthetic pathway for branched-chain amino acids. *Amino Acids* 31, 173–210. <https://doi.org/10.1007/s00726-005-0297-3>
- McCourt, J.A., Pang, S.S., King-Scott, J., Guddat, L.W., Duggleby, R.G., 2006. Herbicide-binding sites revealed in the structure of plant acetohydroxyacid synthase. *Proc. Natl. Acad. Sci.* 103, 569–573. <https://doi.org/10.1073/pnas.0508701103>
- Nakata, P.A., Anderson, J.M., Okita, T.W., 1994. Structure and expression of the potato ADP-glucose pyrophosphorylase small subunit. *J. Biol. Chem.* 269, 30798–807.
- Naveed, Z.A., Bibi, S., Ali, G.S., 2019. The *Phytophthora* RXLR effector *avrblb2* modulates plant immunity by interfering with Ca<sup>2+</sup> signaling pathway. *Front. Plant Sci.* 10, 1–13. <https://doi.org/10.3389/fpls.2019.00374>
- OECD, 1997. Consensus Document on the Biology of *Solanum tuberosum* Subsp. *tuberosum* (potato) <http://www.oecd.org/science/biotrack/46815598.pdf>.
- Oh, S.-K., Young, C., Lee, M., Oliva, R., Bozkurt, T.O., Cano, L.M., Win, J., Bos, J.I.B., Liu, H.-Y., van Damme, M., Morgan, W., Choi, D., Van der Vossen, E.A.G., Vleeshouwers, V.G.A.A., Kamoun, S., 2009. In Planta Expression Screens of *Phytophthora infestans* RXLR Effectors Reveal Diverse Phenotypes, Including Activation of the *Solanum bulbocastanum* Disease Resistance Protein Rpi-blb2. *Plant Cell Online* 21, 2928–2947. <https://doi.org/10.1105/tpc.109.068247>
- Oh, S.K., Kwon, S.Y., Choi, D., 2014. Rpi-blb2-mediated hypersensitive cell death caused by *Phytophthora infestans* AVRblb2 requires SGT1, but not EDS1, NDR1, salicylic acid-, jasmonic acid-, or ethylene-

- mediated signaling. *Plant Pathol. J.* 30, 254–260. <https://doi.org/10.5423/PPJ.OA.03.2014.0027>
- Panstruga, R., Parker, J.E., Schulze-Lefert, P., 2009. SnapShot: Plant Immune Response Pathways. *Cell* 136. <https://doi.org/10.1016/j.cell.2009.02.020>
- Potato Genome Sequencing Consortium, 2011. Genome sequence and analysis of the tuber crop potato. *Nature* 475, 189–95. <https://doi.org/10.1038/nature10158>
- Rosa, C., Kuo, Y.-W., Wuriyanghan, H., Falk, B.W., 2018. RNA Interference Mechanisms and Applications in Plant Pathology. *Annu. Rev. Phytopathol.* 56, 581–610. <https://doi.org/10.1146/annurev-phyto-080417-050044>
- Sharma, S.K., Bolser, D., de Boer, J., Sønderkær, M., Amoros, W., 2013. Construction of reference chromosome-scale pseudomolecules for potato: integrating the potato genome with genetic and physical maps. *Genes Genomes Genet.* 3, 2031–47. <https://doi.org/10.1534/g3.113.007153>
- Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J.D., Higgins, D.G., 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* 7. <https://doi.org/10.1038/msb.2011.75>
- USDA, 2020. Re: USDA Comments on Receipt of a Pesticide Experimental Use Permit Application; EPA-HQ-OPP-2020-0233.
- Witek, K., Jupe, F., Witek, A.I., Baker, D., Clark, M.D., Jones, J.D.G., 2016. Accelerated cloning of a potato late blight-resistance gene using RenSeq and SMRT sequencing. *Nat. Biotechnol.* 34, 656–660. <https://doi.org/10.1038/nbt.3540>













## Justification for Confidential Business Information

September 24, 2021

Certain information associated with the Regulatory Status Review (RSR) for the BG25 potato is deemed by the J.R. Simplot Company (Simplot) to be confidential business information (CBI) and is therefore claimed by Simplot to be exempt from Freedom of Information Act disclosure as defined under 5 U. S. C. Section 552(b) (4).

### **The justifications for the claim of CBI are:**

Sequence of the Insert in BG25: Simplot claims the sequence of the insert in BG25 to be Confidential Business Information (CBI). Simplot has invested considerable time and resources to evaluate literature, transform tissues, and evaluate regenerated plants using many genes/constructs before selecting the relevant event (BG25). Disclosure of the specific sequence of the insert would enable competitors to avoid similar investments of time and resources and allow them to rapidly mimic Simplot's technology and catch up to Simplot in product development, resulting in competitive harm to Simplot. The entire sequence provided is deemed to be CBI.